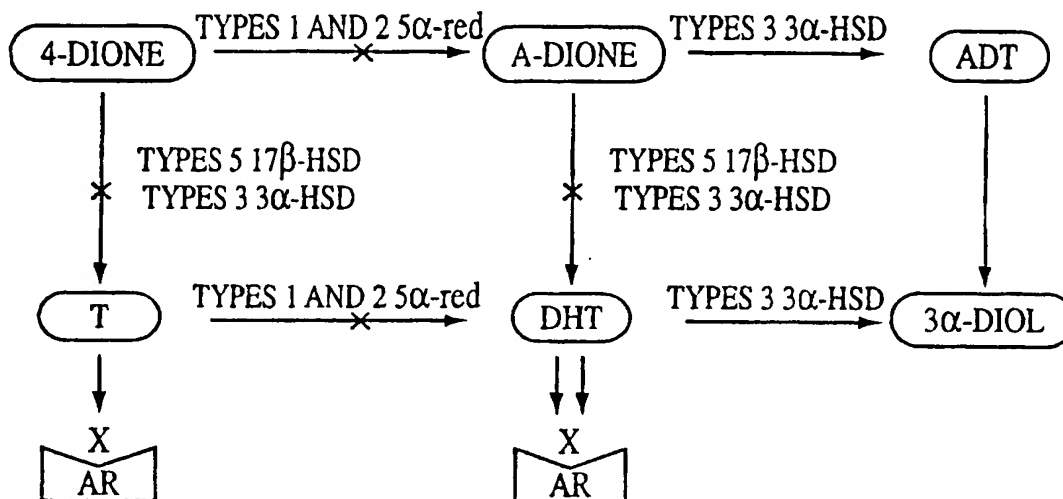




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(54) Title: INHIBITION OF TYPE 3 3 α -HYDROXYSTEROID DEHYDROGENASE

X, INHIBITION TARGET

(57) Abstract

Novel methods of treating and/or inhibiting development of prostatic cancer, benign prostatic hyperplasia, prostatitis, acne, seborrhea, hirsutism or androgenic alopecia utilize inhibitors of type 3 3 α -hydroxysteroid dehydrogenase alone or in combination with other active pharmaceuticals such as inhibitors of type 5 17 β -hydroxysteroid dehydrogenase. Novel inhibitors and pharmaceutical products are also disclosed.

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INHIBITION OF TYPE 3 3 α -HYDROXYSTEROID DEHYDROGENASEFIELD OF THE INVENTION

The present invention relates to methods of treatment of sex steroid-dependent diseases based upon the use of inhibitors of enzymes involved in the biosynthesis of sex steroids from natural precursors. In particular, inhibitors that reduce the natural production of androgens such as testosterone and dihydrotestosterone, are disclosed.

BACKGROUND OF THE RELATED ART

Many androgen-sensitive diseases, i.e. diseases whose onset or progress is aided by androgenic activity, are known. They include but are not limited to prostate cancer, benign prostatic hyperplasia, acne, seborrhea, hirsutism, androgenic alopecia, precocious puberty, adrenal hyperplasia, and polycystic ovarian syndrome. Estrogen sensitive diseases, i.e. diseases whose onset or progress is aided by estrogenic activity are also known. They include but are not limited to breast cancer, endometrial cancer, endometriosis, leiomyoma, and precocious puberty.

Androgenic and estrogenic activity may be suppressed by administering androgen receptor antagonists ("antiandrogens") or estrogen receptor antagonists ("antiestrogens"), respectively. See e.g. WO 94/26767 and WO 96/26201. Androgenic and estrogenic activity may also be reduced by suppressing androgen or estrogen biosynthesis or

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secretions by known methods. See e.g. WO 90/10462, WO 91/00731, WO 91/00733, and WO 86/01105. Type 5 17 β -hydroxysteroid dehydrogenase is described in WO 97/11162.

The molecular cloning and characterization of the human type 3 3 α -hydroxysteroid dehydrogenase from human prostatic cDNA library have been described by Dufort et al., Biochemical and Biophysical Research Communications 228, 474-479 (1996).

Inhibitors of human type 5 17 β -hydroxysteroid dehydrogenase enzyme are disclosed in United States Provisional Patent Application filed in March 11 1998, as serial No 60/077,510.

Effective inhibitors of human type 3 3 α -hydroxysteroid dehydrogenase enzyme or effective inhibitors of both human type 3 3 α -hydroxysteroid dehydrogenase and human type 5 17 β -hydroxysteroid dehydrogenase enzymes are provided by the present invention, as is the discovery that androgen formation can be suppress thereby. The prior art is not believe to have described or suggested that the inhibition of type 3 3 α -hydroxysteroid dehydrogenase may play a beneficial role in reducing the amount of testosterone and dihydrotestosterone available in target tissues.

SUMMARY OF THE INVENTION

It is accordingly an object of the present invention to more selectively and effectively inhibit the conversion of 4-androstene-3,17-dione to testosterone and 5 α -androstane-3,17-dione to dihydrotestosterone using an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase while preferably avoiding inhibition of type 2 or 4 17 β -hydroxysteroid

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dehydrogenases, type 1 3α -hydroxysteroid dehydrogenase, or any other androgen degradation enzyme.

It is another object of the present invention to more selectively and effectively inhibit the conversion of 4-androstene-3,17-dione to testosterone and 5α -androstane-3,17-dione to dihydrotestosterone using an inhibitor of both human type 3 3α -hydroxysteroid dehydrogenase and type 5 17α -hydroxysteroid dehydrogenase while preferably avoiding inhibition of type 2 or 4 17β -hydroxysteroid dehydrogenases, type 1 3α -hydroxysteroid dehydrogenase, or any other androgen degradation enzyme.

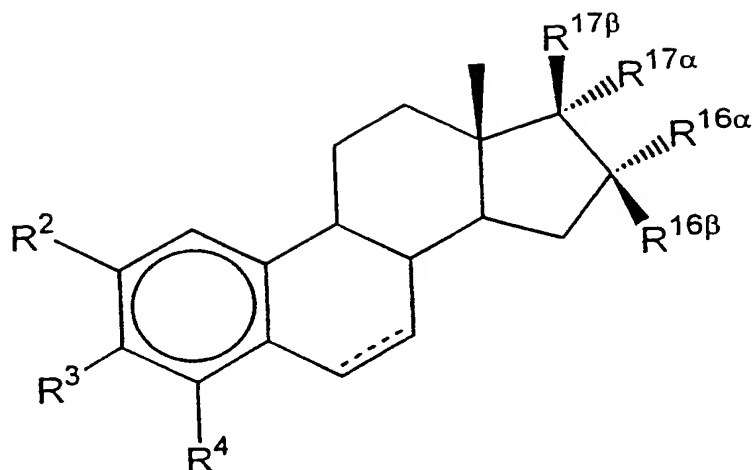
It is another object to provide treatment and prevention regimens for prostate cancer, benign prostatic hyperplasia and prostatitis.

It is another object to provide treatment and prevention regimens for androgen-sensitive skin diseases, particularly acne, seborrhea, hirsutism and androgenic alopecia.

In one embodiment, the invention provides a method of inhibiting conversion of 4-androstene-3,17-dione to testosterone or of 5α -androstane-3,17-dione to dihydrotestosterone in a patient in need of such inhibition comprising administering to said patient a therapeutically effective amount of an inhibitor of human type 3 3α -hydroxysteroid dehydrogenase other than 17-lactone derivative compounds.

In another embodiment, the invention provides a method of inhibiting activity of human type 3, 3α -hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an inhibitor of human type 3 3α -hydroxysteroid dehydrogenase having the following structure:

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wherein the dotted line is an optional pi bond;

wherein R³ is a moiety selected from the group consisting of C₁-C₂₀ alkyloxy, C₁-C₁₀ acyloxy, C₁-C₂₀ alkoxycarbonyloxy, C₁-C₂₀ alkyloxy alkyloxy, hydroxyl, (N-alkyl or -H) carbamate and a moiety transformed in vivo to hydroxyl;

wherein R² and R⁴ are independently selected from the group consisting of hydrogen, cyano, fluoro, chloro, bromo, and nitro (wherein R² and R⁴ are not simultaneously hydrogen).

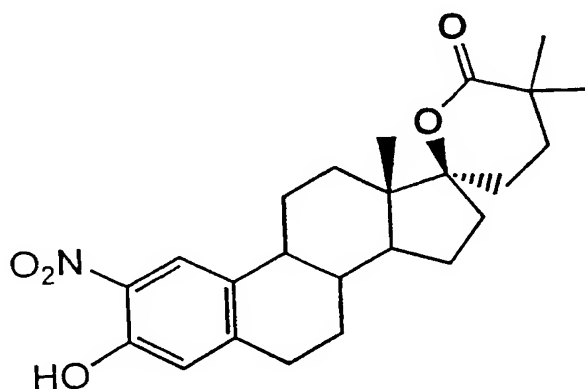
wherein R¹⁷ᵃ is selected from the group consisting of hydrogen, a C₂-C₁₄ carbon moiety substituted by a radical selected from the group consisting of hydrogen, halogen, carboxyl, amido, C₁-C₃ alkoxy and C₁-C₅ alkyl or R¹⁷ᵃ and R¹⁷ᵇ together form a C₅-C₇ lactone ring or is a ketonic oxygen;

wherein R¹⁷ᵇ is hydroxyl, acyloxy, alkoxy, alkenyloxy, (N-alkyl or H) amido; or R¹⁷ᵃ and R¹⁷ᵇ together form a C₅-C₇ lactone ring or is a ketonic oxygen;

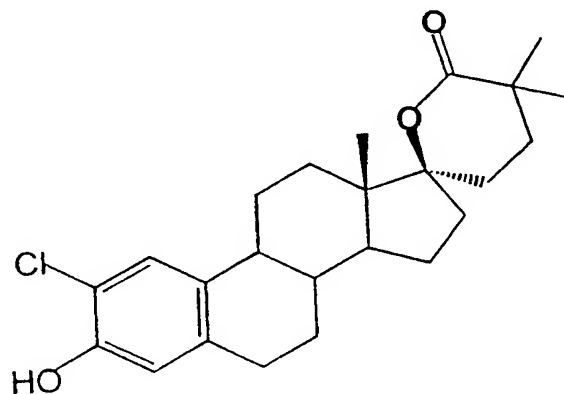
wherein R¹⁶ᵃ and R¹⁶ᵇ are independently selected from the group consisting of hydrogen, lower alkyl, and benzyl, or R¹⁶ᵃ and R¹⁶ᵇ together form a C₅-C₆ cycloalkene.

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In another embodiment, the invention provides a method of inhibiting the activity of human type 3 3α -hydroxysteroid dehydrogenase comprising administering to a patient in need of such inhibition a therapeutically effective amount of an inhibitor of human type 3 3α -hydroxysteroid dehydrogenase selected from the group consisting of:



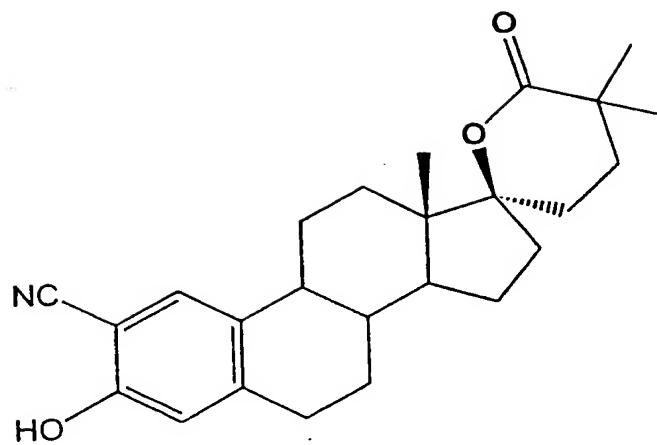
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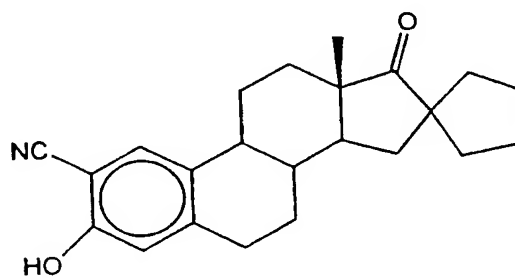
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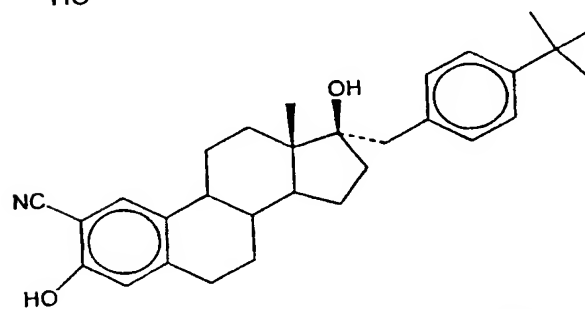
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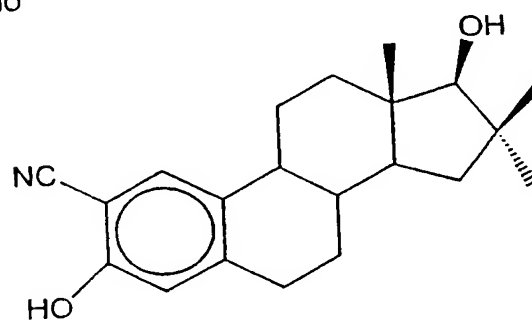
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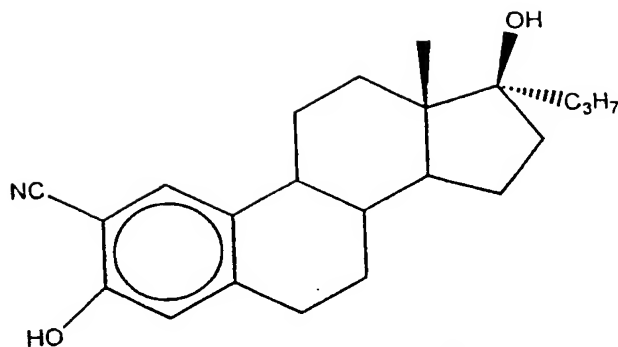


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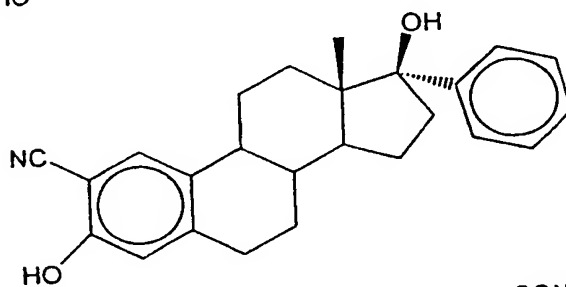


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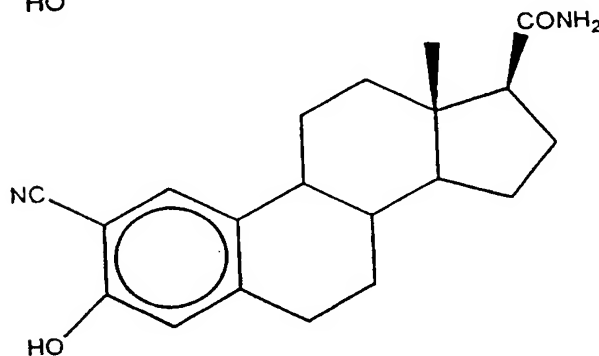
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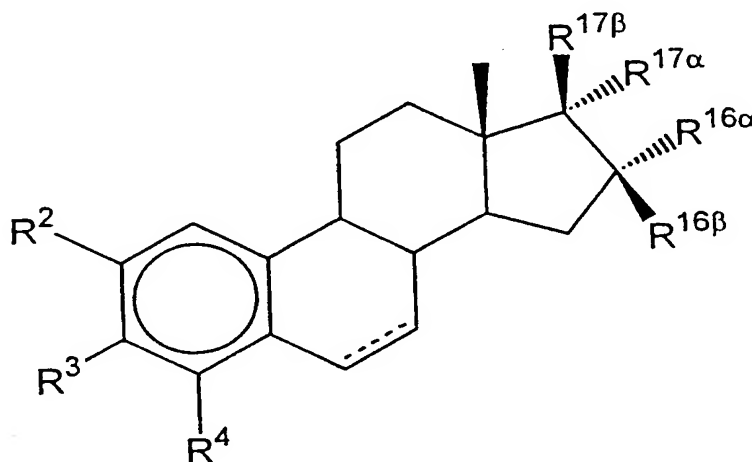
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In another embodiment, the invention provides a method for determining effectiveness of a putative inhibitor of the conversion of 4-androstene-3,17-dione to testosterone and 5 α -androstane-3,17-dione to dihydrotestosterone, comprising measuring activity of type 3 3 α -hydroxysteroid dehydrogenase in the presence of said putative inhibitor and correlating effectiveness to a reduction in said activity relate to activity of said dehydrogenase in the absence of said putative inhibitor.

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In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically effective amount of an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase having the molecular structure:



wherein R³ is a moiety selected from the group consisting of C₁-C₂₀ alkyloxy, C₁-C₁₀ acyloxy, C₁-C₂₀ alkoxycarbonyloxy, C₁-C₂₀ alkyloxy alkyloxy, hydroxyl, (N-alkyl or -H) carbamate and a moiety transformed in vivo to hydroxyl;

wherein R² and R⁴ are independently selected from the group consisting of hydrogen, cyano, fluoro, chloro, bromo, and nitro (wherein R² and R⁴ are not simultaneously hydrogen).

wherein the dotted line is an optional pi bond;

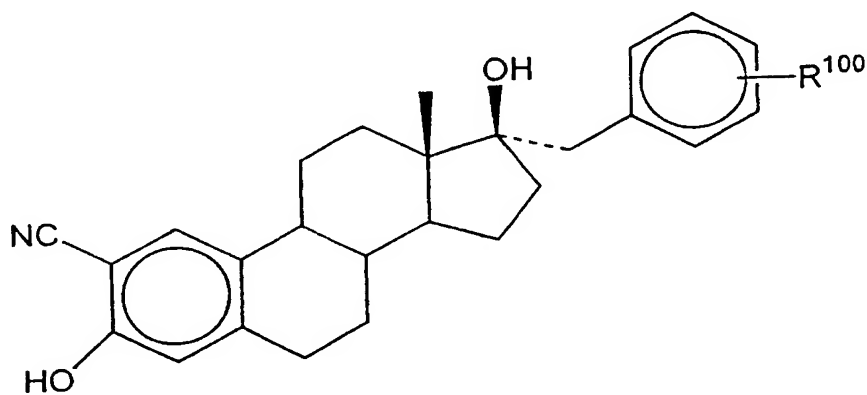
wherein R^{17α} is selected from the group consisting of hydrogen, a C₂-C₁₄ carbon moiety substituted by a radical selected from the group consisting of hydrogen, halogen,

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carboxyl, amido, C₁-C₃ alkoxy and C₁-C₅ alkyl or R^{17a} and R^{17b} together form a C₅-C₇ lactone ring or is a ketonic oxygen;

wherein R^{17b} is selected from the group consisting of hydroxyl, acyloxy, alkoxy, alkenyloxy, (N-alkyl or H) amido; or R^{17a} and R^{17b} together form a C₅-C₇ lactone ring or is a ketonic oxygen;

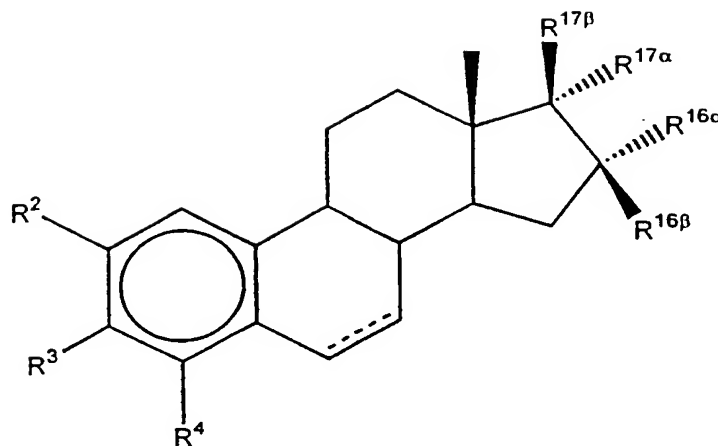
In another embodiment, the invention provides a pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase having the molecular structure:



wherein R¹⁰⁰ is selected from the group consisting of hydrogen, carboxyl, amido, C₁-C₅ alkyl, halo, nitro, hydroxy, and C₁-C₃ alkoxy.

In another embodiment, the invention provides an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase having the molecular structure:

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wherein R^3 is a moiety selected from the group consisting of C_1 - C_{20} alkyloxy, C_1 - C_{10} acyloxy, C_1 - C_{20} alkoxy carbonyloxy, C_1 - C_{20} alkyloxy alkyloxy, hydroxyl; (N-alkyl or -H) carbamate and a moiety transformed in vivo to hydroxyl;

wherein R^2 and R^4 are independently selected from the group consisting of hydrogen, cyano, fluoro, chloro, bromo, and nitro (wherein R^2 and R^4 are not simultaneously hydrogen).

wherein the dotted line is an optional pi bond;

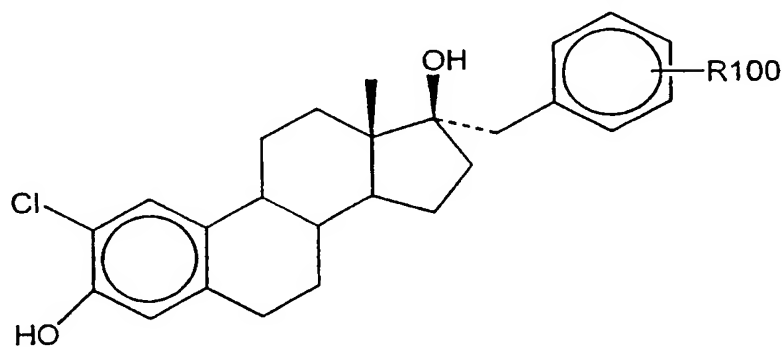
wherein $R^{17\alpha}$ is selected from the group consisting of hydrogen, a C_2 - C_{14} carbon moiety substituted by a radical selected from the group consisting of hydrogen, halogen, carboxyl, amido, C_1 - C_3 alkoxy and C_1 - C_5 alkyl or $R^{17\alpha}$ and $R^{17\beta}$ together form a C_5 - C_7 lactone ring or is a ketonic oxygen;

wherein $R^{17\beta}$ is selected from the group consisting of hydroxyl, acyloxy, alkyloxy, alkenyloxy, (N-alkyl or H) amido; or $R^{17\alpha}$ and $R^{17\beta}$ together form a C_5 - C_7 lactone ring or is a ketonic oxygen;

wherein $R^{16\alpha}$ and $R^{16\beta}$ are independently selected from the group consisting of hydrogen, lower alkyl, and benzyl, or $R^{16\alpha}$ and $R^{16\beta}$ together form a C_5 - C_6 cycloalkene.

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In another embodiment, the invention provides an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase having the molecular structure:



wherein R¹⁰⁰ is selected from the group consisting of hydrogen, carboxyl, amido, C₁-C₅ alkyl, halo, nitro, hydroxy, and C₁-C₃ alkoxy.

In another embodiment, the invention provides a method of treating, or reducing the risk of developing prostate cancer, comprising administering to a patient in need of such treatment or reduction a therapeutically effective amount of an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase other than 17-lactone derivative compounds.

In another embodiment, the invention provides a method of treating, or reducing the risk of developing, benign prostatic hyperplasia comprising administering to a patient in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase other than 17-lactone derivative compounds.

In another embodiment, the invention provides a method of treating, or reducing the risk of developing, prostatitis comprising administering to a patient in need of such

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treatment or reduction, a therapeutically effective amount of an inhibitor of human type 3 3α -hydroxysteroid dehydrogenase.

In another embodiment, the invention provides a method of treating or reducing the risk of developing acne, seborrhea, hirsutism or androgenic alopecia comprising administering to a said patient, in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of human type 5 17β -hydroxysteroid dehydrogenase activity of human type 3 3α -hydroxysteroid dehydrogenase other than by administering a 17-lactone derivative compound.

The inhibitors of the invention are used for preventing and/or treating certain diseases, discussed herein, whose onset or progress is stimulated by androgenic activity. One of the more surprising results of our laboratory work is the discovery that type 3 3α -HSD which is known for its catalytic activity of reactions affecting the 3 position of steroids has now been shown by Applicants to catalyze reactions affecting the 17 position. This discovery that type 3 3α -HSD participates in the formation of testosterone and DHT from androstenedione and androstane-3,17-dione permits enhanced suppression of biosynthesis of these two important androgens by suppressing this new biosynthetic pathway that Applicants have discovered. It has been found that type 3 3α -hydroxysteroid dehydrogenase displays activity similar to that of 17β -hydroxysteroid dehydrogenase (catalyzing the conversion of 4-androstenedione-3,17-dione to testosterone and 5α -androstane-3,17-dione to dihydrotestosterone), inhibitors which suppress the 17β -hydroxysteroid dehydrogenase activity of type 3 3α -hydroxysteroid dehydrogenase, with or without combination with inhibitors of type 5 17β -hydroxysteroid dehydrogenase and/or inhibitors of 5α -reductase diminish the production of androgens catalyzed by these

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enzymes. Because androgens formed by reactions catalyzed by these enzymes are precursors to estrogens, the invention also has applicability to diseases whose onset or progress is aided by estrogenic activity.

With respect to all of the dosages recommended herein, the attending clinician should monitor individual patient response, and adjust dosage accordingly.

A patient in need of treatment or reducing the risk of onset of a given disease is one who has either been diagnosed with such disease or one who is susceptible to acquiring such disease.

Except where otherwise stated, the preferred dosage of the active compounds of the invention is identical for both therapeutic and prophylactic purposes. The dosage for each active component discussed herein is the same regardless of which particular disease is being treated (or prevented).

As used in the methods of medical treatment of methods of reduction of risk of onset of disease herein, an "inhibitor of type 3 3α -hydroxysteroid dehydrogenase" means a compound whose IC_{50} of inhibition for the enzyme in question (computed in the same manner as described in connection with Table 1 herein) is no higher than 200 nM. It is preferred that IC_{50} of such inhibitor be no higher than 50 nM, most preferably lower than 10 nM. It is also preferred that undesirable inhibition of 3α -HSD type 1 and 17β -HSD type 2 be less than 90% at $3 \cdot 10^{-7}M$, preferably less than 80%, and most preferably less than 70%. In some embodiments, it is preferred that androgenicity be less than 100% of stimulation of

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Shionogi cells at a concentration of $10^{-7}M$, preferably less than 50%, most preferably less than 20%.

Where two or more different active agents are discussed as part of a combination therapy herein (e.g. an enzyme inhibitor and an antiandrogen), a plurality of different compounds are administered rather than a single compound having multiple activities.

Except where otherwise noted or where apparent from context, dosages herein refer to weight of active compounds unaffected by pharmaceutical excipients, diluents, carriers or other ingredients, although such additional ingredients are desirably included, as shown in the examples herein. Any dosage form (capsule, tablet, injection or the like) commonly used in the pharmaceutical industry is appropriate for use herein, and the terms "excipient", "diluent" or "carrier" include such non-active ingredients as are typically included, together with active ingredients in such dosage forms in the industry. For example, typical capsules, pills, enteric coatings, solid or liquid diluents or excipients, flavorants, preservatives, or the like may be included.

As stated herein, the term of hydrocarbon moiety includes but are not limited to straight or branched alkyl, straight or branched alkenyl, straight or branched alkynyl, phenyl, phenylalkyl, phenylalkenyl, phenylalkynyl.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic biosynthesis pathway of active androgens in the human prostate.

Figure 2 shows 17 β -HSD and 3 α -HSD activity in intact 293 cells (ATCC CRL 1573) stably transfected with type 3 3 α -HSD, in culture. Cells stably transfected with type 3 3 α -HSD were seeded into 24-well plates at a density of 10⁵ cells/well. 0.1 μ M of [¹⁴C]-labeled 4-dione and [¹⁴C]-labeled DHT were added to freshly changed culture medium to assess the 17 β -HSD activity of type 3 3 α -HSD enzyme [transformation of 4-dione to testosterone (\square)] and 3 α -HSD activity of type 3 3 α -HSD enzyme [transformation of DHT to 3 α -diol (O)] activity of the transfected enzyme, respectively. Non transfected cells were used as control. After incubation for the indicated time periods, the media were collected and extracted, and assayed as described herein "Enzymatic assay for types 1, 2, 3, and 5 17 β -HSD and types 1 and 3 3 α -HSD".

Figures 3a to 3c show paraffin sections of normal human skin immunostained with antibody to type 3 3 α -HSD. The presence of type 3 3 α -hydroxysteroid dehydrogenase can be seen in:

- a) epithelium and fibroblast
- b) hair follicle
- c) sudoriferous glands.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Prostate cancer is a disease of the prostatic epithelium while benign prostatic hyperplasia (BPH) mainly involves the stromal compartment of the prostate. Prostatitis, although more common in the prostatic epithelium, can be found in both areas of the prostate.

Applicants' recent results show that type 5 17β -hydroxysteroid dehydrogenase (type 5 17β -HSD) and type 3 3α -hydroxysteroid dehydrogenase (type 3 3α -HSD) are present in the prostatic epithelium, mainly the basal cells, thus transforming androstenedione into testosterone by the pathways shown in Figure 1. Such testosterone then diffuses into the luminal epithelial cells which are androgen-dependent, and where prostate cancer grows. Concerning the stromal compartment, type 3 3α -HSD and type 5 17β -HSD are mainly found in the fibroblasts which are distributed among the muscle cells. It is believed that growth factors secreted by the fibroblasts stimulate the surrounding cells, thus leading to BPH. The presence of estrogen receptors in these fibroblasts in the stroma probably provides the basis for the role of estrogens as well as androgens in BPH. While Prostate cancer, however, is essentially only an androgen-sensitive disease.

In the prior art, type 3 3α -HSD was known to convert DHT into androstane- 3α , 17β -diol, an inactive metabolite. Applicants have recently discovered the surprising role of 3α -HSD in catalyzing the formation of testosterone and DHT from androstenedione and androstenedione, respectively (See figure 1). The advantage of inhibiting type 3 3α -HSD to suppress formation of testosterone and DHT is believed to substantially outweigh any reduction in androgen catabolism that might result when type 3 3α -HSD is inhibited.

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The presence of numerous other enzymes which are essential for the formation of androgens in the prostatic tissues suggest the likelihood that combination therapies discussed herein (e.g., use of other inhibitors(s) of enzymatic activity, antiandrogens and/or castration) will lead to superior results relative to use of one active agent alone. This is especially believed true of those combinations that affect disease by two or more separate mechanisms (e.g., inhibiting two or more different synthetic pathways, inhibiting androgen formation in combination with blocking access to androgen receptors, etc.).

Figure 1 applies to each of prostate cancer, benign prostatic hyperplasia, and prostatitis, although the cell types are different between prostate cancer and BPH. In prostate cancer, type 5 17β -HSD and type 3 3α -HSD are mainly present in one cell type (basal cells) while 5α -reductases are present in the luminal cells, which are located just above the basal cells, thus permitting diffusion of testosterone from the basal to the luminal cells and then conversion into the more potent androgen DHT. For the fibroblasts located in the stromal compartment, the transformation of androstenedione to testosterone and then to DHT takes place in the same cells.

Type 1 3α -HSD is not present in a significant amount in the prostate but is mainly a liver enzyme. Inhibitors used in the invention (e.g., inhibitors of type 3 3α -HSD, inhibitors of type 5 17β -HSD, inhibitors of 5α -reductase, etc.) preferably have little or no inhibitory effect on type 1 3α -HSD which beneficially acts to inactivate androgens. Thus, Applicants prefer to avoid the inhibition of type 1 3α -HSD, when practicing the invention, so as not to delay the inactivation of androgens by the hepatic tissue.

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We have found that the human type 3 3α -hydroxysteroid dehydrogenase which transforms 5α -androstane-3,17-dione and dihydrotestosterone to androsterone and androstane- 3α , 17β -diol, respectively, also transforms 4-androstene-3,17-dione to testosterone and 5α -androstane-3,17-dione to dihydrotestosterone. In prostatic tissue, the expression of human type 3 3α -hydroxysteroid dehydrogenase is much higher than the expression of the human type 5 17β -hydroxysteroid dehydrogenase enzyme, thus implying that a significant proportion of androgens in the prostate are formed by this pathway (Figure 1).

In one of preferred embodiments, inhibition of androgen formation, as illustrated in Figure 1, is performed by an efficient blockade of dihydrotestosterone (DHT) production with an effective inhibition of both, 5α -reductase and type 5 17β -hydroxysteroid dehydrogenase activities.

There are two types of 5α -reductase. Both types are expressed in the prostate, type 2 5α -reductase, however, is expressed at a higher level. A Merck product, Proscar (finasteride, MK-906), inhibits mostly type 2 5α -reductase.

Compound GI 198745 (17β -{N-2,5-bis(trifluoromethyl) phenyl}carbamoyl-4-aza- 5α -androst-1-en-3-one) produced by Glaxo Wellcome, and EM-503 (17β -(N,benzoyl,N-phenyl)amino-4-methyl-4-aza-androstane-3-one), a compound of Endorecherche, inhibit efficiently both human types 1, and 2 5α -reductase, thus offering the more efficient possibility of a blockade of DHT formation.

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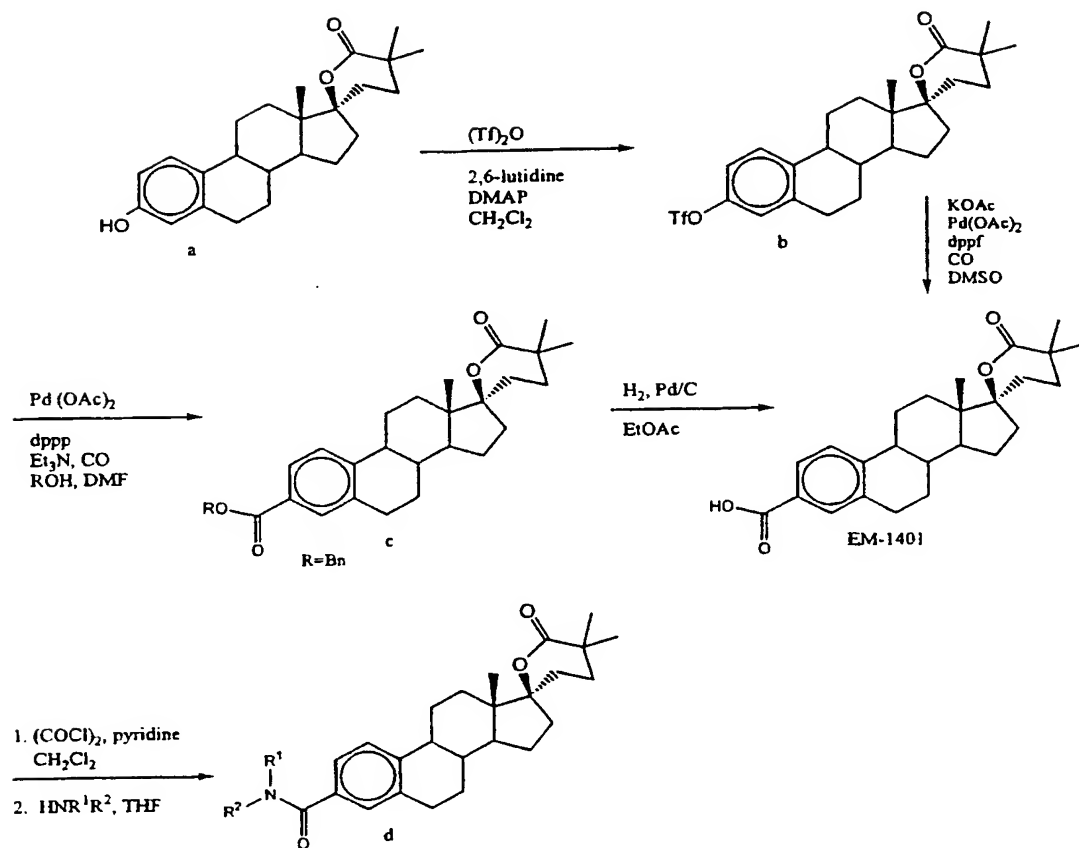
Inhibition of 5 α -reductase activity, however, does increase testosterone (T) levels. Although weaker than DHT, T possesses also an androgenic effect that will keep the prostate growing to a variable extent.

In order to achieve a more efficient blockade of androgen formation, it is useful to also inhibit the 17 β -hydroxysteroid dehydrogenase activity that converts 4-dione to T or A-dione to DHT. This activity is catalyzed in the prostate by type 5 17 β -HSD, and surprisingly by type 3 3 α -HSD. We have already developed a highly efficient inhibitor for type 5 17 β -HSD whose molecular structure and synthesis are set forth below:

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Synthesis of EM-1401, EM-1404

Scheme A



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3-trifluoromethanesulfonyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (b). Under argon atmosphere, a solution of compound a (500 mg, 1.35 mmol), 2,6-lutidine (0.355 mL, 3.05 mmol) and 4-dimethylaminopyridine (33 mg, 0.27 mmol) in dry dichloromethane (25 mL) was cooled at 0 °C, treated with trifluoromethanesulfonic anhydride (0.308 mL, 1.83 mmol) and stirred for 45 min. The reaction mixture was quenched with water and extracted with dichloromethane. The organic phase was washed with 2% hydrochloric acid, saturated sodium bicarbonate and water, dried over magnesium sulfate, filtered, and evaporated. The crude oil was purified by flash chromatography (hexanes-ethyl acetate 49-1 to hexanes-ethyl acetate 4-1) to provide trifluoromethanesulfonate b (EM-1399) (540 mg, 80%): IR (CHCl₃) 2957, 2872, 1711, 1490, 1426, 1248, 1214, 1141, 926, 846, 621 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 1.03 (s, 3H), 1.28 (s, 3H), 1.29 (s, 3H), 1.35-2.40 (m, 17H), 2.88 (m, 2H), 6.98 (s, 1H), 7.02 (d, J=8 Hz, 1H), 7.33 (d, J=8.7 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 14.32, 23.22, 25.48, 25.80, 26.89, 27.57, 27.68, 29.37, 31.49, 31.80, 34.69, 37.72, 38.46, 43.66, 47.10, 48.59, 93.43, 116.54, 118.08, 120.80, 121.07, 127.05, 139.31, 140.43, 147.46, 177.70.

3-carboxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (EM-1401). Method A: A mixture of compound b (560 mg, 1.12 mmol), potassium acetate (440 mg, 4.48 mmol), palladium acetate (12.6 mg, 0.056 mmol), and 1,1'-bis(diphenylphosphino)ferrocene (125 mg, 0.255 mmol) in dimethyl sulfoxide (20 mL) was purged with carbon monoxide for 20 min and stirred over under a carbon monoxide balloon at 80 °C over a 3 h period. The reaction mixture was diluted with 0.5 N hydrochloric acid and extracted with dichloromethane. The organic phase was washed with water, dried over magnesium sulfate, filtered, and evaporated. The reaction mixture was purified by flash chromatography (dichloromethane-methanol 19-1 to

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dichloromethane-methanol 4-1) to provide the carboxylic acid EM-1401 (300 mg, 68%): IR (KBr) 2937, 2872, 1718, 1676, 1388, 1314, 1230, 1180, 1160 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3 + CD_3OD) δ 0.75 (s, 3H), 1.01 (s, 6H), 1.10-2.17 (m, 17H), 2.65 (m, 2H), 7.09 (d, $J=8.1$ Hz, 1H), 7.48 (s, 1H), 7.51 (d, $J=8.5$ Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3 + CD_3OD) δ 13.71, 22.75, 24.98, 25.27, 26.65, 26.87, 28.76, 30.84, 31.46, 34.21, 37.33, 38.22, 43.84, 46.74, 93.92, 124.84, 126.52, 127.32, 129.91, 136.31, 144.94, 168.70, 178.97.

3-alkoxycarbonyl-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-

oxo)tetrahydropyran (c). A mixture of compound b, triethylamine (3.25 equiv), palladium acetate (0.07 equiv), 1,3-bis(diphenylphosphino)propane (0.06 equiv), and alcohol (1.5 equiv to large excess) in DMF (10% W/V) was purged with carbon monoxide for 20 min and stirred under a carbon monoxide balloon at 90 °C over a 16 h period. The reaction mixture was cooled at room temperature, diluted with water and extracted with dichloromethane. The organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The reaction mixture was purified by 3 flash chromatographies (2 times with benzene-acetone 4-1 and hexanes-ethyl acetate 7-3) to provide compound c (e.g., EM-1398, R=benzyl, 70%): IR (CHCl_3) 2938, 1716, 1293, 1262, 1177, 1152, 1130, 1109, 732 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 1.02 (s, 3H), 1.28 (s, 3H), 1.29 (s, 3H), 1.34-1.41 (m, 17H), 2.91 (m, 2H), 5.35 (s, 2H), 7.33-7.45 (m, 6H), 7.79 (s, 1H), 7.83 (d, $J=8.1$ Hz, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.39, 23.28, 25.55, 25.74, 27.14, 27.64, 27.75, 29.25, 31.56, 31.93, 34.75, 37.77, 38.56, 44.34, 47.16, 48.82, 66.42, 93.50, 125.34, 126.90, 127.45, 128.05, 128.10, 128.52, 130.23, 136.22, 136.81, 145.49, 166.55, 177.75.

3-carboxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (EM-1401). Method B: A mixture of compound c (350 mg, 0.72 mmol) and 10% palladium on

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activated carbon (50 mg) in ethyl acetate (40 mL) was stirred under an hydrogen balloon over a 3 h period. The reaction mixture was filtered on celite and evaporated. The crude mixture was purified by flash chromatography (dichloromethane-THF 19-1 to dichloromethane-THF 3-1) to provide the carboxylic acid **EM-1401** (240 mg, 84%). A sample was recrystallized in methanol-THF (the characterization was described previously).

3-carboxamido-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (d). Under argon atmosphere, a solution of **EM-1401** and pyridine (15 equiv) in dry dichloromethane (1.6% W/V) was cooled at 0 °C, treated with oxalyl chloride (6 equiv) and stirred for 0.5 h. The reaction mixture was allowed to reach room temperature and stirred over a 4 h period. The reaction mixture was evaporated, dissolved in dry THF (1.6% W/V), cooled at 0 °C, treated with 10 equiv of amine and stirred for 15 min. The reaction mixture was quenched with water, extracted with dichloromethane, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (hexanes-acetone 19-1 to hexanes-acetone 3-2) to provide compound **d** (e.g., **EM-1404**, R¹=R²=H, 65%): IR (CHCl₃) 3433, 3350, 2941, 2873, 1702, 1664, 1611, 1388, 1310, 1159 cm⁻¹; ¹H NMR (300 MHz, CDCl₃ + CD₃OD) δ 0.73 (s, 3H), 0.99 (s, 6H), 1.10-2.16 (m, 17H), 2.64 (m, 2H), 7.08 (d, *J*=8.0 Hz, 1H), 7.30 (s, 1H), 7.32 (d, *J*≈9 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃ + CD₃OD) δ 13.69, 22.72, 24.96, 25.27, 26.64, 26.84, 28.78, 29.09, 30.81, 31.44, 34.19, 37.31, 38.27, 43.72, 46.74, 93.92, 124.20, 124.93, 127.70, 130.00, 136.45, 143.88, 170.63, 178.96.

Type 5 17β-HSD and type 3 3α-HSD share 85.5% amino acid identity. The high primary structure homology between type 5 17β-HSD and type 3 3α-HSD could explain a minor

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17 β -HSD activity found in type 3 3 α HSD activity. However, since type 3 3 α -HSD is expressed at a much higher level than type 5 17 β -HSD, the unexpected 17 β -HSD activity contributed by type 3 3 α -HSD plays a significant role in the prostate. Inhibition of type 3 3 α -HSD activity is thus necessary to have an efficient blockade of androgen formation (Figure 1).

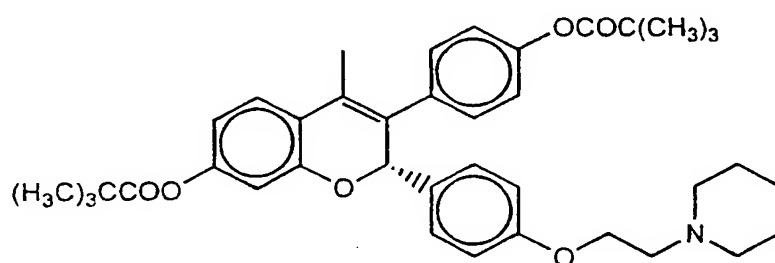
Inhibitors of type 3 3 α -hydroxysteroid dehydrogenase may, in accordance with the invention, be utilized alone or as part of a combination therapy with other strategies (listed below) which have beneficial effects on androgen-sensitive diseases through different mechanisms, thus providing synergistic combinations. These combination therapies include in addition to type 3 inhibitors of 3 α -hydroxysteroid dehydrogenase (and in some embodiments in combination with an inhibitor of type 5 17 β -hydroxysteroid dehydrogenase) one or more of the following strategies:

Strategy 1: Suppression of ovarian or testicular hormonal secretion by chemical or surgical castration. This approach is useful for the treatment of diseases which respond adversely to estrogen or androgen, respectively. When surgical or chemical castration is utilized, chemical castration is preferred utilizing either an LHRH-agonist, an LHRH antagonist and/or an inhibitor of type 3 17 β -hydroxysteroid dehydrogenase (which as discussed herein catalyzes some testicular androgen formation). Suitable LHRH agonists are reported in US Patent 4,659,695, but any LHRH agonist showing the ability to induce chemical castration can be used since they all act through the same mechanisms as originally described (Labrie et al., J. Androl. 1: 209-228, 1980). Dosages are known in the art. Some suitable LHRH antagonists are reported in U.S. Patent 4,666,885 but any LHRH antagonist is acceptable, if used according to the recommendation of the manufacturer.

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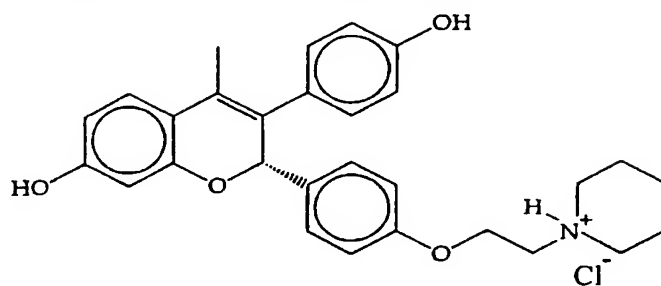
Strategy 2: Utilizing androgen or estrogen receptor antagonists ("antiandrogens" or "antiestrogens") to prevent activation of androgen or estrogen receptors by androgens or estrogens, respectively. Strategy 2 is useful against diseases that respond adversely to androgenic or estrogenic activity, respectively. Antiandrogens, and dosages therefor, are known in the art (e.g. Flutamide (N-[4-nitro-3-(trifluoromethyl)phenyl]-2-methyl propanamide) at a dosage of 250 mg, 2 or 3 times a day, Nilutamide at a dosage of 150 mg/day, Casodex at a dosage of 50 to 750 mg/day.

When antiestrogens are used in accordance with the invention, either alone or as part of one of the combination therapies described herein, the attending clinician should, at least initially, use the dosages recommended by the manufacturer. However, the attending clinician should monitor individual patient response and metabolism and adjust patient dosage accordingly. Indeed, that will be true of all of the strategies discussed herein and all of the active ingredients used in any of the combination therapies of the invention. One preferred antiestrogen is EM-800 reported in PCT/CA96/00097 (WO 96/26201) The molecular structure of EM-800 is:



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Another preferred antiestrogen of the invention is EM-01538 :



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Other preferred SERMs of the invention include Tamoxifen ((Z)-2-[4-(1,2-diphenyl-1-butenyl)]-N,N-dimethylethanamine) (available from Zeneca, UK), Toremifene (available from Orion-Farmos Pharmaceuticla, Finland, or Schering-Plough), Droloxifene and CP-336,156 (cis-1R-[4'-pyrrolidino-ethoxyphenyl]-2S-phenyl-6-hydroxy-1,2,3,4-tetrahydronaphthalene D-(-)-tartrate salt) (Pfizer Inc., USA), Raloxifene (Eli Lilly and Co., USA), LY 335563 and LY 353381 (Eli Lilly and Co., USA), Iodoxifene (SmithKline Beecham, USA), Levormeloxifene (3,4-trans-2,2-dimethyl-3-phenyl-4-[4-(2-(2-(pyrrolidin-1-yl)ethoxy)phenyl]-7-methoxychroman) (Novo Nordisk, A/S, Denmark) which is disclosed in Shalmi et al. WO 97/25034, WO 97/25035, WO 97/25037, WO 97/25038 ; and Korsgaard et al. WO 97/25036), GW5638 (described by Willson et al., Endocrinology, 138(9), 3901-3911, 1997) and indole derivatives (disclosed by Miller et al. EP 0802183A1) and TSE 424 developed by Wyeth Ayers (USA) and disclosed in JP10036347 (American home products corporation) and nonsteroidal estrogen derivatives described in WO 97/32837.

There are two types of 5α -reductase. Both types are expressed in the prostate, type 2 5α -reductase, however, is expressed at a higher level. A Merck product, Proscar (finasteride, MK-906), inhibits mostly type 2 5α -reductase.

Compound GI 198745 (17β -{N-2,5-bis(trifluoromethyl) phenyl}carbamoyl-4-aza- 5α -androst-1-en-3-one) produced by Glaxo Wellcome, and EM-503 (17β -(N,benzoyl,N-phenyl)amino-4-methyl-4-aza-androstane-3-one), a compound of Endorecherche, inhibit efficiently both human types 1, and 2 5α -reductase, thus offering an efficient possibility of a blockade of DHT formation.

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Strategy 3: Suppression of conversion of the androgen testosterone to the more potent androgen dihydrotestosterone (DHT) by inhibiting the activity of testosterone 5 α -reductase (e.g. by administering Proscar, available from Merck Sharp and Dohme Canada, at the recommended dosage). Any other potent 5 α -reductase inhibitor can be used. The dosage used can be 2 to 20 mg daily orally. The dosage should be the one recommended by the manufacturer. Strategy 3 is useful against diseases that respond adversely to androgenic activity.

Strategy 4: Utilizing an aromatase inhibitor to reduce estrogen production. Strategy 4 is useful against diseases that respond adversely to estrogenic activity or estrogen receptor-mediated exacerbation of the type of androgen-sensitive diseases that are also estrogen-sensitive diseases (e.g. benign prostatic hyperplasia). Aromatase inhibitors (and antiestrogens) may also be used to reduce unwanted estrogenic effects that result from increased estrogenic levels that may occur during some treatments of androgen-dependent diseases. When aromatase inhibitors are used in accordance with the invention, either alone or as part of one of the combination therapies described herein, the attending clinician should initially use the dosage recommended by the manufacturer. When administered orally, the dosage which is usually effective to provide the desired serum levels is between 1.0 mg and 20 mg of active ingredient per day per 50 kg of body weight. For example, Arimidex (Zeneca) is taken at the oral dose of 1 mg daily. However, the attending clinician should monitor individual patient response and metabolism and adjust specific patient dosage accordingly. Some aromatase inhibitors include, for example, molecular structures set forth in US patent 5,227,375. Aromatase inhibition may also be achieved, for example, by administering Arimidex (2,2'-[5-(1H-1,2,4-triazol-1-ylmethyl)-1,3-phenylene bis (2-methylpropiononitrile)) available from Zeneca, UK, at a dosage of 1

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mg/day. Any other aromatase inhibitor can be used according to the recommendations of the manufacturer.

In general, for both androgen-sensitive diseases and estrogen-sensitive diseases, simultaneous treatment with inhibitors of sex steroid biosynthesis inhibitors (inhibitors of enzymes which catalyze one or more steps of estrogen or androgen biosynthesis or biosynthesis of estrogen or androgen precursors), and with estrogen receptor antagonists and/or androgen receptor antagonists, are believed to have additive rather than redundant effect because they are acting in a beneficial manner by a different mechanism. Likewise, the activity of two different enzyme inhibitors (enzymes which catalyze one or more different steps of sex steroid biosynthesis) are believed to provide additive effect, especially where the inhibitors affect more than one synthetic pathway. Such an approach is believed to achieve a more complete effect.

The type 3 3α -hydroxysteroid dehydrogenase inhibitors and inhibitor of type 5 17β -hydroxysteroid dehydrogenase of the invention may be used in any combination with any of the strategies 1-4 above whose effect (increasing or decreasing androgenic or estrogenic activity) is consistent with a desirable effect on the disease in question. With that in mind, set forth below are a list of representative diseases which may be treated, or the risk of which may be reduced, in accordance with the present invention. Beneath each disease, are indicated several preferred therapies or combination therapies for treatment, or risk reduction, of that particular disease. However, these combinations may be supplemented using one or more of the four strategies listed above, limited only by whether a particular disease responds favorably or adversely to estrogenic activity and/or to androgenic activity.

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- A) Prostate cancer (responds adversely to androgenic activity)
1. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase
 2. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase
 3. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + LHRH-agonist (or antagonist).
 4. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + inhibitor of type 3 17β -hydroxysteroid dehydrogenase.
 5. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + inhibitor of type 3 17β -hydroxysteroid dehydrogenase + LHRH agonist (or antagonist).
 6. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + LHRH-agonist (or antagonist) + antiandrogen
 7. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + inhibitor of type 3 17β -hydroxysteroid dehydrogenase + antiandrogen.
 8. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + inhibitor of type 3 17β -hydroxysteroid dehydrogenase + LHRH agonist (or antagonist) + antiandrogen.
 9. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiandrogen + 5α -reductase inhibitor + LHRH agonist (or antagonist).

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10. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + inhibitor of type 5 17 β -hydroxysteroid dehydrogenase + LHRH agonist + 5 α -reductase inhibitor.
11. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + inhibitor of type 5 17 β -hydroxysteroid dehydrogenase + inhibitor of type 3 17 β -hydroxysteroid dehydrogenase + 5 α -reductase inhibitor.
12. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + inhibitor of type 5 17 β -hydroxysteroid dehydrogenase + inhibitor of type 3 17 β -hydroxysteroid dehydrogenase + antiandrogen + 5 α -reductase inhibitor.
13. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + inhibitor of type 5 17 β -hydroxysteroid dehydrogenase + inhibitor of type 3 17 β -hydroxysteroid dehydrogenase + LHRH agonist (or antagonist) + antiandrogen + 5 α -reductase inhibitor.

B) Benign prostatic hyperplasia (responds adversely to both androgenic activity and estrogenic activity)

1. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase.
2. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + antiestrogen or aromatase inhibitor.
3. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + antiandrogen.
4. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + antiandrogen + 5 α -reductase inhibitor + antiestrogen or aromatase inhibitor.
5. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + 5 α -reductase inhibitor.
6. Inhibitor of type 3 3 α -hydroxysteroid dehydrogenase + antiandrogen + 5 α -reductase inhibitor.

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7. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + 5α -reductase inhibitor + antiestrogen or aromatase inhibitor.
8. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase.
9. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiestrogen or aromatase inhibitor.
10. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiandrogen.
11. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiandrogen + 5α -reductase inhibitor + antiestrogen or aromatase inhibitor.
12. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + 5α -reductase inhibitor.
13. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiandrogen + 5α -reductase inhibitor.
14. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + 5α -reductase inhibitor + antiestrogen or aromatase inhibitor.

C) Prostatitis (responds adversely to androgenic activity)

1. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + antiandrogen.
2. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + 5α -reductase inhibitor.
3. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + antiandrogen + 5α -reductase inhibitor.

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4. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase.
5. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiandrogen.
6. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + 5α -reductase inhibitor.
7. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiandrogen + 5α -reductase inhibitor.

D) Acne, seborrhea, hirsutism, and androgenic alopecia (responds adversely to androgenic activity)

1. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase.
2. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase.
3. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + antiandrogen.
4. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + antiandrogen.
5. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of 5α -reductase.
6. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of type 5 17β -hydroxysteroid dehydrogenase + inhibitor of 5α -reductase.
7. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + antiandrogen + inhibitor of 5α -reductase.
8. Inhibitor of type 3 3α -hydroxysteroid dehydrogenase + inhibitor of 17β -hydroxysteroid dehydrogenase + antiandrogen + inhibitor of 5α -reductase.

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When type 3 3α -hydroxysteroid inhibitors are used in accordance with the invention, either alone or as part of one of the combination therapies described herein, the attending clinician desirably will target patient serum concentration of the type 3 inhibitor between 0.5 ng/ml and 100 ng/ml, preferably between 1 ng/ml and 20 ng/ml, and most preferably between 1 ng/ml and 10 ng/ml. Serum concentration may be measured by LC/MS. When administered orally, the dosage which is usually effective to provide the desired serum levels is between 1.0 mg and 1,000 mg of active ingredient per day per 50 kg of body weight, preferably between 10 mg and 500 mg and most preferably between 10 mg and 100 mg. However, dosage should vary with the bioavailability of the chosen inhibitor and with individual patient response. For example, when EM-01645, or EM-01667-C are chosen, oral dosage is preferably between 5 mg and 500 mg per day per 50 kg body weight, more preferably between 10 mg/day and 300 mg/day, for example between 20 mg/day and 100 mg/day. The attending clinician should monitor individual patient response and serum levels, if judged appropriate, and adjust patient dosage accordingly. When administered by injection, a lesser dosage is usually appropriate, e.g. 10 mg to 100 mg per day per 50 kg of body weight.

When type 5 17β -hydroxysteroid inhibitors are used in accordance with the invention, as part of one of the combination therapies described herein, the attending clinician desirably will target patient serum concentration of the type 5 inhibitor between 0.5 ng/ml and 100 ng/ml, preferably between 1 ng/ml and 20 ng/ml, and most preferably between 1 ng/ml and 10 ng/ml. Serum concentration may be measured by LC/MS. When administered orally, the dosage which is usually effective to provide the desired serum levels is between 1.0 mg and 1,000 mg of active ingredient per day per 50 kg of body weight, preferably between 10 mg and 500 mg and most preferably between 10 mg and 100 mg. However,

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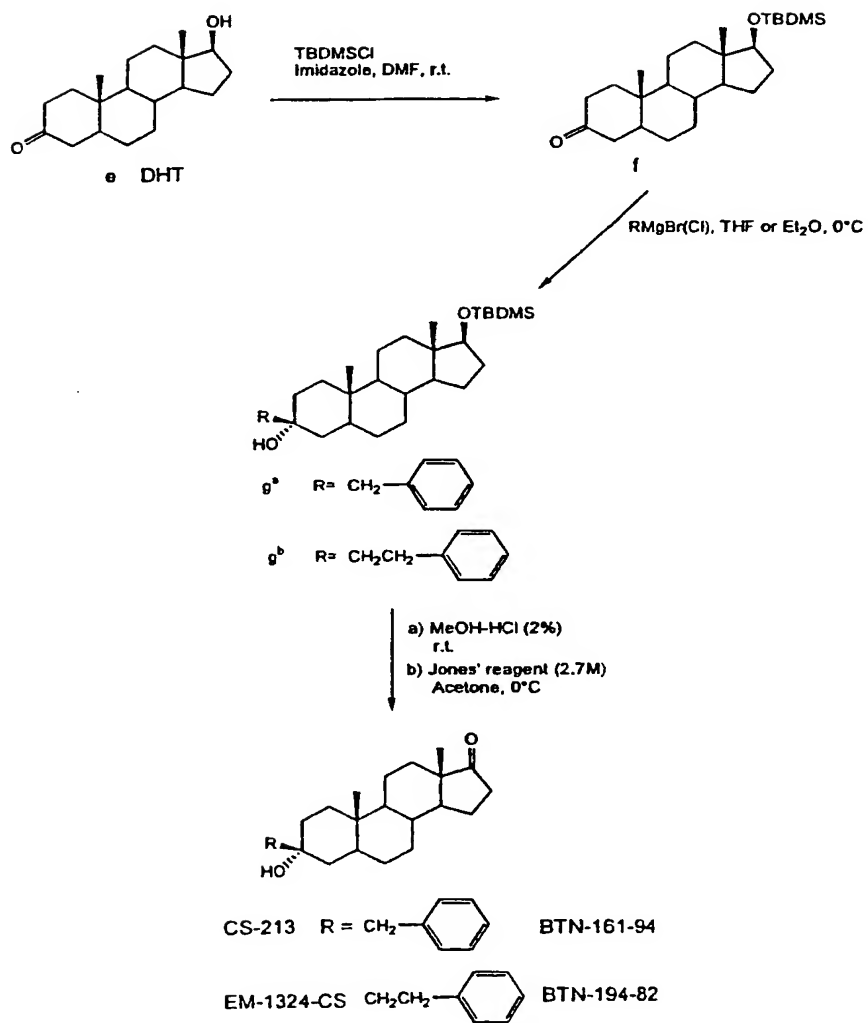
dosage should vary with the bioavailability of the chosen inhibitor and with individual patient response. For example, when EM-1404 are chosen, oral dosage is preferably between 5 mg and 500 mg per day per 50 kg body weight, more preferably between 10 mg/day and 300 mg/day, for example between 20 mg/day and 100 mg/day. The attending clinician should monitor individual patient response and metabolism (serum levels, if judged appropriate) and adjust patient dosage accordingly. When administered by injection, a lesser dosage is usually appropriate, e.g. 10 mg to 100 mg per day per 50 kg of body weight.

When type 3 17β -hydroxysteroid inhibitors are used in accordance with the invention, as part of one of the combination therapies described herein, the attending clinician desirably will target patient serum concentration of the type 3 inhibitor between 0.5 ng/ml and 100 ng/ml, preferably between 1 ng/ml and 20 ng/ml and most preferably between 1 ng/ml and 10 ng/ml. When administered orally, the dosage is preferably between 1.0 mg and 1,000 mg of active ingredient per day per 50 kg of body weight, preferably between 5 mg and 500 mg and most preferably between 10 mg and 100 mg. However, the attending clinician should monitor individual patient response and metabolism and adjust patient dosage accordingly. Synthesis of such an inhibitor is described below.

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Synthesis of type 3 17 β -HSD inhibitors

Scheme B



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Protection of the 17 β -alcohol with TBDMS. To a solution of dihydrotestosterone (DHT, e) (5g, 17.2 mmol) in DMF was added imidazole (6 eq.) and TBDMSCl (5 eq.). The reaction was stirred overnight at room temperature. The mixture was poured onto ice and filtered. The resulting white precipitate was washed with water, dried over phosphorous pentoxide under reduced pressure for 24 h. A 85 to 90% yield was obtained.

17 β -[(*tert*-butyldimethylsilyl)oxy]-5 α -androstane-3-one (f). White solid; IR (KBr) ν 1719 (C=O, ketone); ^1H NMR (CDCl_3) δ -0.001 and 0.005 (s, 6H, $\text{Si}(\text{CH}_3)_2$), 0.71 (s, 3H, CH_3 -18), 0.87 (s, 9H, $\text{SiC}(\text{CH}_3)_3$), 1.01 (s, 3H, CH_3 -19), 3.54 (t, J = 8.2 Hz, 1H, CH-17); ^{13}C NMR (CDCl_3) δ -4.80 and -4.47, 11.41, 11.52, 18.11, 21.13, 23.56, 25.87, 28.98, 30.94, 31.36, 35.54, 35.78, 37.13, 38.21, 38.65, 43.36, 44.74, 46.84, 50.55, 54.15, 81.79, 212.03.

Alkylation of the carbonyl at position 3. To a solution of compound f (500 mg, 1.23 mmol) in dry THF (100 mL) at 0°C was added dropwise 3 eq. of commercially available Grignard's reagent, in dry THF. The mixture was allowed to react for 3 h at 0°C, then left over night at room temperature. A solution of saturated NH_4Cl was added and the crude product was extracted with EtOAc. The organic phase was washed with a saturated NaCl solution, dried over MgSO_4 and evaporated under reduced pressure. The 3 β -alkylated stereoisomer was easily separated from the 3 α -alkylated stereoisomer by flash chromatography on silica gel, using a mixture of hexanes and ethyl acetate as eluent. When the Grignard's reagent was generated *in situ* as in the case of ethylphenyl magnesium bromide, 5 eq. was prepared, by a well-known procedure, using the corresponding bromide, activated magnesium and iodide. The steroid was then dissolved in dry diethyl ether and added dropwise to the solution of reagent. The yields obtained were around 60% for the two stereoisomers.

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3 β -benzyl-17 β [(*tert*-butyldimethylsilyl)oxy]-3 α -hydroxy-5 α -androsterane (**g^a**). White solid (24%); IR (KBr) ν 3585 and 3460 (OH, alcohol); ^1H NMR (CDCl_3) δ 0.002 and 0.009 (s, 6H, $\text{Si}(\text{CH}_3)_2$), 0.69 (s, 3H, CH_3 -18), 0.75 (s, 3H, CH_3 -19), 0.88 (s, 9H, $\text{SiC}(\text{CH}_3)_3$), 2.71 (s, 2H, CH_2Ph), 3.54 (t, $J = 8.2$ Hz, 1H, CH-17), 7.20 to 7.34 (5H, Ph); ^{13}C NMR (CDCl_3) δ -4.82 and -4.50 ($\text{SiC}(\text{CH}_3)_3$), 11.25, 11.40, 18.08, 20.62, 23.50, 25.85, 28.41, 30.91, 31.62, 33.27, 33.81, 35.60, 35.84, 37.19, 40.10, 40.84, 43.30, 50.43, 50.69, 54.43, 71.22, 81.82 (C-17), 126.37, 128.09 (2X), 130.56 (2X), 137.06.

3 α -hydroxy-3 β -(phenylethyl)-17 β [(*tert*-butyldimethylsilyl)oxy]-5 α -androsterane (**g^b**). White solid (38%); IR (film) ν 3447 (OH, alcohol); ^1H NMR (CDCl_3) δ 0.018 and 0.025 (s, 6H, $\text{Si}(\text{CH}_3)_2$), 0.71 (s, 3H, CH_3 -18), 0.78 (s, 3H, CH_3 -19), 0.89 (s, 9H, $\text{SiC}(\text{CH}_3)_3$), 2.73 (m, 2H, Ph- CH_2), 3.56 (t, $J = 8.1$ Hz, 1H, CH-17), 7.18 to 7.31 (5H, Ph); ^{13}C NMR (CDCl_3) δ -4.77 and -4.46 ($\text{Si}(\text{CH}_3)_3$), 11.28, 11.44, 18.12 ($\text{SiC}(\text{CH}_3)_3$), 20.67, 23.54, 25.89 ($\text{SiC}(\text{CH}_3)_3$), 28.52, 29.60, 30.97, 31.66, 33.31, 33.92, 35.66, 36.04, 37.25, 40.03, 41.05, 43.35, 46.47, 50.76, 54.55, 71.50 (C-3), 81.86 (C-17), 125.68, 128.38 (4X), 142.82.

Procedure for hydrolysis of TBDMS group and oxidation of the resulting alcohol. The silylated ether was dissolved in a methanolic solution of HCl (2%, v/v) and the resulting mixture was stirred at room temperature for 3h. Water was then added and MeOH evaporated under vacuum. The resulting white precipitate was submitted to Jones' oxidation without purification. To a stirred solution of crude alcohol in acetone at 0°C, Jones' reagent (2.7M chromic acid solution) was added dropwise. After 30 to 45 minutes, the reaction was completed. Isopropanol and water were added and acetone was removed in vacuo. The remaining aqueous layer was extracted with EtOAc. The combined organic

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phases were washed with brine, dried over MgSO₄, filtered and evaporated under reduced pressure. The purification was done on silica gel, using HPLC grade solvents, EtOAc and hexanes as eluents.

3 β -benzyl-3 α -Hydroxy- -5 α -androstane-17-one (CS-213). White solid (88% for the two steps); IR (KBr) ν 3408 (OH, alcohol), 1732 (C=O, ketone); ¹H NMR (CDCl₃) δ 0.75 (s, 3H, CH₃-19), 0.84 (s, 3H, CH₃-18), 2.69 (s, 2H, CH₂Ph), 7.18 to 7.32 (5H, Ph); ¹³C NMR (CDCl₃) δ 11.18, 13.78, 20.20, 21.71, 28.16, 30.79, 31.52, 33.18, 33.70, 35.64, 35.79, 35.88, 39.97, 40.69, 47.75, 50.39, 51.41, 54.22, 71.12, 126.40, 128.09 (2X), 130.51 (2X), 136.93, 221.27

3 α -hydroxy-3 β -phenylethyl-5 α -androstane-17-one (EM-1324-CS). White solid (82% for the two steps); IR (film) ν 3486 (OH, alcohol), 1737 (C=O, ketone); ¹H NMR (CDCl₃) δ 0.79 (s, 3H, CH₃-19), 0.86 (s, 3H, CH₃-18), 2.71 (m, 2H, Ph-CH₂), 7.18 to 7.30 (5H, Ph); ¹³C NMR (CDCl₃) δ 11.21, 13.82, 20.26, 21.76, 28.26, 29.54, 30.87, 31.58, 33.27, 33.80, 35.10, 35.84, 36.07, 39.89, 40.90, 46.43, 47.80, 51.49, 54.35, 71.42, 125.69, 128.31 (2X), 128.39 (2X), 142.70, 221.31.

All of the active ingredients used in any of the therapies discussed herein may be formulated in pharmaceutical compositions which include one or more of the other active ingredients. Alternatively, they may each be administered separately but sufficiently simultaneous in time so that a patient eventually has elevated blood levels or otherwise enjoys the benefits of each of the active ingredients (or strategies) simultaneously. In some preferred embodiments of the invention, for example one or more active ingredients are to be formulated in a single pharmaceutical composition. In other embodiments of the invention, a kit is provided which includes at least two separate containers wherein, the contents of at least one container differs in whole or in part from the contents of at least

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one other container with respect to active ingredients contained therein. Two or more different containers are used in these combination therapies of the invention. Combination therapies discussed herein also include use of one active ingredient of the combination in the manufacture of a medicament for the treatment (or prevention) of the disease in question where the treatment or prevention further includes the other active ingredient(s) or strategy of the combination. Some embodiments of the methods of treating or preventing disease discussed herein, utilize the specific type 5 17β -hydroxysteroid dehydrogenase inhibitor and/or type 3 3α -hydroxysteroid dehydrogenase inhibitors discussed herein (i.e. the molecular structures discussed herein).

LHRH agonists and LHRH antagonists may be used interchangeably to suppress either testicular or ovarian hormonal secretions by known techniques, except where preferences are otherwise stated herein. It is desired that activation of glucocorticoid receptors be minimized when administering the active ingredients of the invention. Inhibitors of type 3 17β -hydroxysteroid dehydrogenase may be used to provide advantages similar to those provided by LHRH agonists or antagonists.

PREFERRED INHIBITORS OF TYPE 3 **3α -HYDROXYSTEROID DEHYDROGENASE**

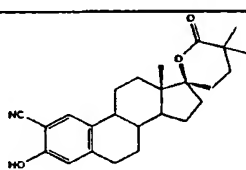
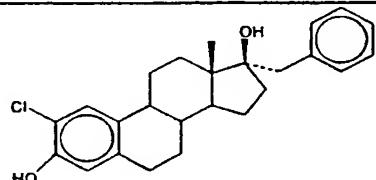
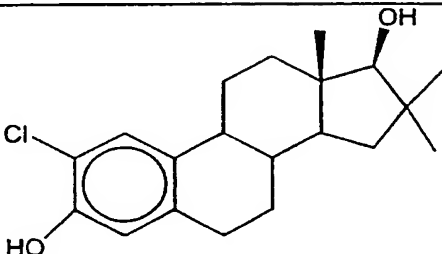
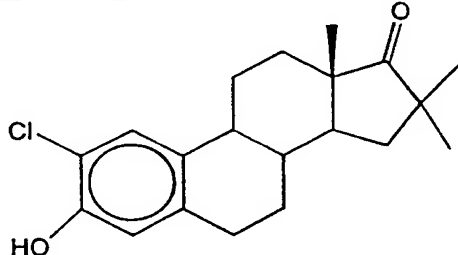
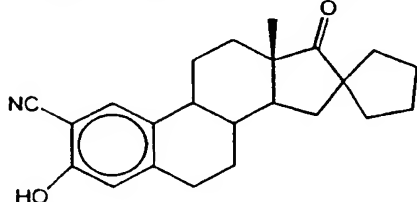
Set forth in the tables below are lists of compounds which we have found to be useful as inhibitors of type 3 3α -hydroxysteroid dehydrogenase. The tables also include in many instances further tests of a particular compound on other important parameters such as androgenic and antiandrogenic activity and the effect of a compound on androgen receptors, proliferation of androgen-sensitive cells, and other effects more fully explained

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below. In tables below that do not include a "prime" (') in their table number, details of molecular structure of preferred inhibitors (or comparison compounds) are set forth. The corresponding tables with a "prime" (') in their table number shows information about the functional efficacy of each tested compound. The numbers in the column headings correspond to a description at the end of all of the tables regarding what information is reported in each column and how it is determined. Entries left blank are not yet determined.

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Table 1

Laboratory Name	STRUCTURE
EM-01645	
EM-2330	
EM-1832	
EM-1831	
EM-1834	

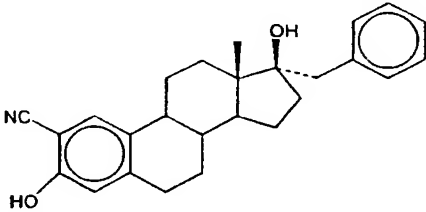
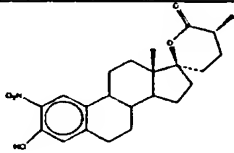
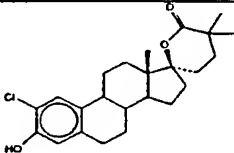
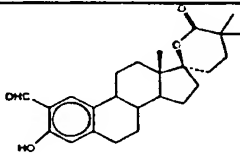
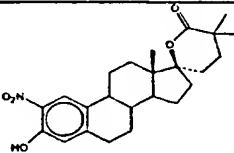
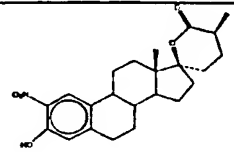
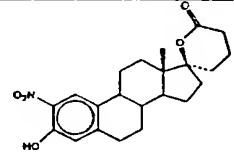
Laboratory Name	STRUCTURE
EM-1836	
EM-1131	
EM-01667-C	
EM-01621	
EM-1125-CS	
EM-1126	
EM-1124	

Table 1'

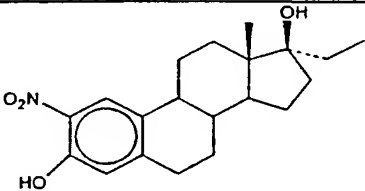
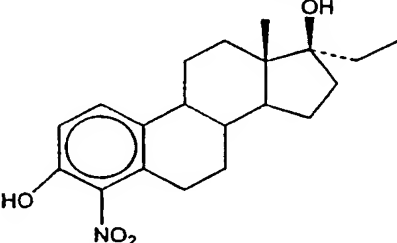
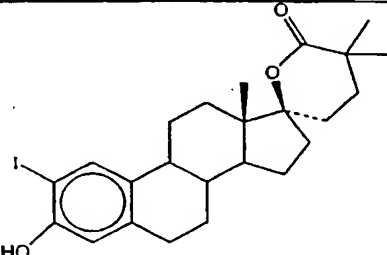
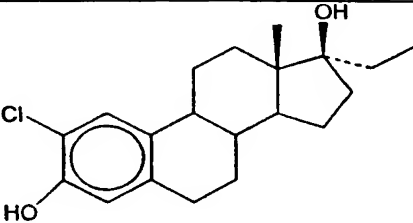
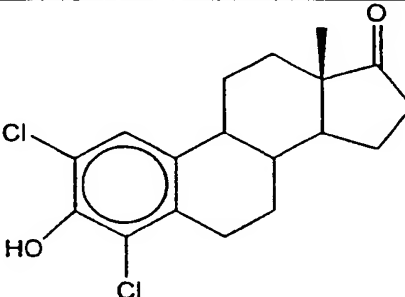
Name	1 Oral Bioavailability AUC 0-7h (ng/mL.h)	2 Inhibition Type 3 IC ₅₀ (nM) (% Inh. (3.10 ⁻⁷ 3.10 ⁻⁶))	3 Inhibition Type 1 IC ₅₀ (nM)	4 Inhibition Type 2 IC ₅₀ (nM)	5 Inhibition Type 3 IC ₅₀ (nM)	6 Inhibition Type 1 IC ₅₀ (nM)	7 Inhibition Type V IC ₅₀ (nM)	8 Shionogi Activity Basal E-7 E-8 DHT E-7 E-8 [IC ₅₀ (nM)]		10 ZR-75-1 Activity Basal E-7 E-8 3E-8E-9 3E-8 E-8	11	12 % Inhibition Androgen Receptor (E-7 E-5) E-8 E-6	13 % Inhibition Progesterone Receptor (E-7 E-6) E-8 E-6	14 % Inhibition Glucocorticoid Receptor (E-7 E-5) E-8 E-6	15 % Inhibition Estrogen Receptor (E-7 E-5) E-8 E-6
ENI-01645	52±3	<1.0		(35 38)		4.2±1	4.5					0 0 0	0 3		4 5
ENI-2330		1.3±0.1		(24 32)		592	(10 37)	65, 65	12 -38			0 3	0 3 1	8 0	27
ENI-1832		(97 98)				(62 91)									
ENI-1831		(96 96)				(76 94)									
ENI-1834		(99 97)				(72 83)									
ENI-1836		(96 97)													
ENI-1131	124±27	77 84		(21 70)		99 100	3	0 0 -15 -47			0 0 2	1 2 10	0 0 5	3	
ENI-01667-C	152±18	95 98		(56 83)		50±6		6 -3 -9 -43							
		(91 96)													
		8±1													

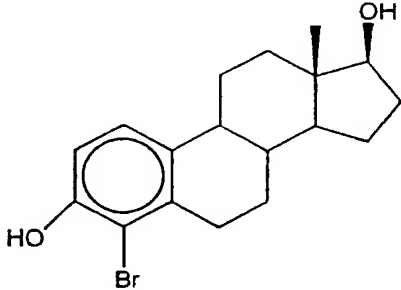
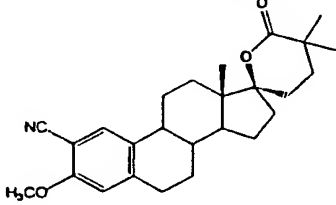
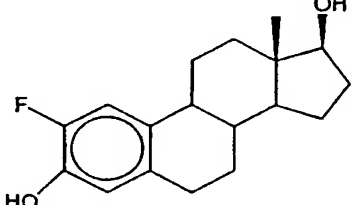
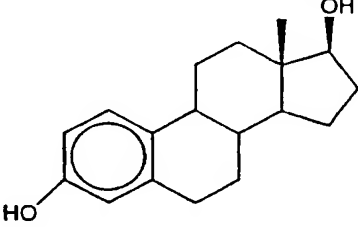
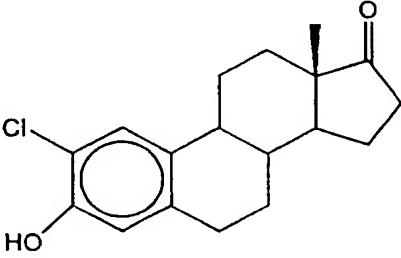
Name	1	2	3	4	5	6	7	Shionogi Activity			ZR-75-1 Activity		12	13	14	15			
								Basal E-7 E-6	OHIT E-7 E-6 [IC50 (nM)]	Basal E2 3E-8E-6 3E-8 E-6	% Inhibition Androgen Receptor (E-7 E-5) E-8 E-6	% Inhibition Progesterone Receptor (E-7 E-5) E-8 E-6					% Inhibition Glucocorticoid Receptor (E-7 E-5) E-8 E-6		
EM-01621	Oral Bioavailability (ng/mL, h)	Inhibition Type 3 IC50 (nM) (% inh. (3.10-7,3.10-6)	Inhibition Type 1 IC50 (nM)	Inhibition Type 2 IC50 (nM)	Inhibition Type 3 IC50 (nM)	Inhibition Type 1 IC50 (nM)	Inhibition Type V IC50 (nM)	69	56	147	60	1	3	0	0	0	6		
EM-1125-CS	95 ± 6.5	(68 95) 118±9 1.84± 0.3		(53 68) (58 83)		83	2	3	-10	-30 -43	10 34 0 .0	2	1	5	7	0	2	2	
EM-1126	ND	78 88		(26 59)		84	92	0	-8	0 -49	0 -89 0 0	2	10	5	14	0	0	1	0
EM-1124	5.1 ± 1 (8h)	89 91				90	94	0	-11	0 -65	0 12 0 0	1	7	1	20	1	1	0	0

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Table 2

Laboratory Name	STRUCTURE
EM-01678	
EM-01666	
EM-01692	
EM-01729	
EM-01762	

Laboratory Name	STRUCTURE
EM-01801	 <chem>Oc1cc(Br)ccc1[C@H]2CC[C@H]3[C@@H](O)CC[C@]3(C)[C@H]2C</chem>
EM-01807	 <chem>COc1cc(C#N)ccc1[C@H]2CC[C@H]3[C@@H](C(=O)C(C)(C)C)CC[C@]3(C)[C@H]2C</chem>
EM-01812	 <chem>Oc1cc(F)ccc1[C@H]2CC[C@H]3[C@@H](O)CC[C@]3(C)[C@H]2C</chem>
EM-01813 (estradiol)	 <chem>Oc1ccc2ccccc2c1[C@H]3CC[C@H]4[C@@H](O)CC[C@]4(C)[C@H]3C</chem>
EM-1430	 <chem>Oc1cc(Cl)ccc1[C@H]2CC[C@H]3[C@@H](C=O)CC[C@]3(C)[C@H]2C</chem>

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Table 2'

Name	1	2	3	4	5	6	7	Shionogi Activity			ZR-75-1 Activity		12	13	14	15
								Basal E-7 E-8	DHT E-7 E-8 [IC ₅₀ (nM)]	Basal E ₂ 3E-8E-6 3E-8 E-8						
	Oral Bioavailability (ng/mL.h)	Inhibition Type 3 IC ₅₀ (nM) (% inh. At 13.10 ⁻⁷ 3.10 ⁻⁶)	Inhibition Type 1 IC ₅₀ (nM)	Inhibition Type 2 IC ₅₀ (nM)	Inhibition Type 3 IC ₅₀ (nM)	Inhibition Type 1 IC ₅₀ (nM)	Inhibition Type V IC ₅₀ (nM)						% Inhibition Androgen Receptor (E-7 E-5) E-8 E-8	% Inhibition Progesterone Receptor (E-7 E-5) E-8 E-8	% Inhibition Glucocorticoid Receptor (E-7 E-5) E-8 E-8	% Inhibition Estrogen Receptor (E-7 E-5) E-8 E-8
EN1-01678		(96 95)		(56 83)			(63 91)	-4 7	-60 -86				0 19	0 66	3 13	0 0
EN1-01666		(51 81)		(54 83)			(0 20)	-25 5	-26 -93				1 19	16 23	7 3	3 18
EN1-01692		(40 39)		(37 25)			(88 95)						0 2	0 0	0 2	0 3
EN1-01729		(85 91)		(68 87)			(16 62)	99 137	-19 -15				0 28	0 76	3 78	2 27
EN1-01762		(88 96)		(25 56)			(9 27)	-4 30	-64 -52				0 2	10 2	2 2	0 0
EN1-01801		(32 79)														
EN1-01807		(36 59)														
EN1-01812		(89 95)														

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Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Oral Bioavailability (ng/mL.h)	Inhibition Type 3 IC ₅₀ (nM) (% inh. At 3.10 ⁻⁷ 3.10 ⁻⁶)	Inhibition Type 1 IC ₅₀ (nM)	Inhibition Type 2 IC ₅₀ (nM)	Inhibition Type 3 IC ₅₀ (nM)	Inhibition Type 1 IC ₅₀ (nM)	Inhibition Type V IC ₅₀ (nM)	Shionogi Activity		ZR-75-1 Activity		% Inhibition Androgen Receptor (E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶	% Inhibition Progesterone Receptor (E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶	% Inhibition Glucocorticoid Receptor (E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶	% Inhibition Estrogen Receptor (E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶
ENI-01813		(52 84)				(14 32)		Basal E ⁻⁷ E ⁻⁶	DHT E ⁻⁷ E ⁻⁸ (IC ₅₀ (nM))	Basal E ₂ 3E ⁻⁸ E ⁻⁶ 3E ⁻⁹ E ⁻⁸		(E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶	(E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶	(E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶	(E ⁻⁷ E ⁻⁵) E ⁻⁸ E ⁻⁶
ENI-1430		(93 92)		(69 73)		(66 93)									

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5 LEGENDS TO TABLES

10 In column 1, the oral bioavailability of preferred type 3 3α -hydroxysteroid dehydrogenase inhibitors, expressed in ng/mL.h, was determined as described below in " *In Vivo* Assays of Bioavailability of Human type 3 3α -hydroxysteroid dehydrogenase inhibitors". Higher number are desirable. ND means that a determination was not done.

15 In column 2, the inhibition of human type 3 3α -hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC_{50} in nM) is reported. The manner in which IC_{50} was determined is described infra in " II- Enzymatic assay for types 1, 2, 3 and 5 17β -HSD and types 1 and 3 3α -HSD. Blank means that a determination was not done. In parentheses is reported the percentage of inhibition of enzymatic activity by the inhibitor at 3.10^{-7} and 3.10^{-6} M.

25 In column 3, the inhibition of human type 1 17β -hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC_{50} in nM) is reported. The manner in which IC_{50} was determined is described in " II- Enzymatic assay for types 1, 2, 3 and 5 17β -HSD and types 1 and 3 3α -HSD". Higher numbers of IC_{50} are desirable. Blank means that a determination was not done. In parentheses is reported the percentage of inhibition of enzymatic activity by the inhibitor at 3.10^{-7} and 3.10^{-6} M.

30

- 5 In column 4, the inhibition of human type 2 17 β -hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in " II- Enzymatic assay for types 1, 2, 3 and 5 17 β -HSD and types 1 and 3 3 α -HSD". Higher
10 numbers of IC₅₀ are desirable. Blank means that a determination was not done. In parentheses is reported the percentage of inhibition of enzymatic activity by the inhibitor at 3.10⁻⁷ and 3.10⁻⁶ M.
- 15 In column 5, the inhibition of human type 3 17 β -hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in " II- Enzymatic assay for types 1, 2, 3 and 5 17 β -HSD and types 1 and 3 3 α -HSD". Lower
20 numbers of IC₅₀ are desirable. Blank means that a determination was not done. In parentheses is reported the percentage of inhibition of enzymatic activity by the inhibitor at 3.10⁻⁷ and 3.10⁻⁶ M.
- 25 In column 6, the inhibition of human type 1 3 α -hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition of enzymatic activity (IC₅₀ in nM) is reported. The manner in which IC₅₀ was determined is described in " II- Enzymatic assay for types 1, 2, 3 and 5 17 β -HSD and types 1 and 3 3 α -HSD". Higher
30 numbers of IC₅₀ are desirable. Blank means that a determination was not done. In parentheses is reported the percentage of inhibition of enzymatic activity by the inhibitor at 3.10⁻⁷ and 3.10⁻⁶ M.

5

In column 7, the inhibition of human type 5 17β -hydroxysteroid dehydrogenase activity expressed by the concentration which produce 50% of inhibition (IC_{50} in nM) is reported (centered numbers). The manner in which IC_{50} was determined is described in " II- Enzymatic assay for types 1, 2, 3 and 5 17β -HSD and types 1 and 3 3α -HSD". Lower numbers for IC_{50} are desirable. When IC_{50} was not determined, the percentage of inhibition is reported in parentheses at $3.10^{-7}M$ (left number) and $3.10^{-6}M$ (right number). In parentheses is reported the percentage of inhibition of enzymatic activity by the inhibitor at 3.10^{-7} and $3.10^{-6} M$.

In column 8, the androgenic activity of preferred type 3 3α -hydroxysteroid dehydrogenase inhibitors expressed as the percentage of stimulation of proliferation of Shionogi cells at concentrations of $10^{-7} M$ (left number) and $10^{-6} M$ (right number) of inhibitor. The manner in which the stimulation is determined is described in " III- Androgenic/Antiandrogenic Activity ". Lower numbers are desirable. ND means that a determination was not done.

In column 9, the antiandrogenic activity of preferred type 3 3α -hydroxysteroid dehydrogenase inhibitors expressed by the concentration which produce 50% of inhibition (IC_{50} in nM) of DHT-induced proliferation of Shionogi cells is reported (bracketed centered numbers). The percentage of inhibition of DHT-induced proliferation of Shionogi cells at concentrations of $10^{-7}M$ (left number) and $10^{-6}M$ (right

5 number) of inhibitor is also reported. The manner in which the inhibition is determined is described in "III- Androgenic/Antiandrogenic Activity". Lower numbers are desirable. ND means that a determination was not done.

10 In column 10, the estrogenic activity of preferred type 3 3α -hydroxysteroid dehydrogenase inhibitors expressed as the percentage of stimulation of the proliferation of ZR-75-1 cells at concentrations of 10^{-7} M (left number) and 10^{-6} M (right number) of inhibitor. The manner in which the stimulation is determined is described in "IV-
15 Estrogenic/Antiestrogenic Activity" Lower numbers are desirable. ND means that a determination was not done.

In column 11, the antiestrogenic activity of preferred type 3 3α -hydroxysteroid dehydrogenase inhibitors expressed as percentage of
20 inhibition of E_2 -induced proliferation of ZR-75-1 cells at a concentrations of 10^{-7} M (left number) and 10^{-6} (right number) of inhibitor is reported. The manner in which the inhibition is determined is described in "IV- Estrogenic/Antiestrogenic Activity". Lower numbers are desirable. ND means that a determination was not done.

25 In column 12, the binding on androgen receptor expressed as percentage of inhibition of the binding of $[^3H]R1881$ at the concentration of 10^{-8} M (stared number at 10^{-7} M) (left number) and 10^{-6} M (stared number at 10^{-5} M) (right number) of inhibitor is reported. The manner in which the
30 percentage of inhibition is determined is described in "V-Androgen Receptor (AR) Assays". Lower numbers are desirable.

5 In column 13, the binding on progesterone receptor expressed as percentage of inhibition of the binding of [^3H]R5020 at the concentration of 10^{-8}M (stared number at 10^{-7}M) (left number) and 10^{-6}M (stared number at 10^{-5}M) (right number) of inhibitor is reported. The manner in which the percentage of inhibition is determined is described in "VI- Progesterone Receptor Assay". Lower numbers are desirable.

15 In column 14, the binding on glucocorticoid receptor expressed as percentage of inhibition of the binding of [6,7- $^3\text{H}^*(\text{N})$]-dexamethasone at the concentration of 10^{-8}M (stared number at 10^{-7}M) (left number) and 10^{-6}M (stared number at 10^{-5}M) (right number) of inhibitor is reported. The manner in which the percentage of inhibition is determined is described in " VII- Glucocorticoid Receptor Assay ".

20 In column 15, the binding on estrogen receptor expressed as percentage of inhibition of the binding of [^3H]E $_2$ at the concentration of 10^{-8}M (stared number 10^{-7}) (left number) and 10^{-6}M (stared number 10^{-5}) (right number) of inhibition is reported. The manner in which the percentage of inhibition is determined is described in " VIII-Estrogen Receptor (ER) Assay ".

25

EFFICACY OF THE PREFERRED INHIBITORS

I- In Vivo Assays of Bioavailability of Human type 3 3α -hydroxysteroid dehydrogenase inhibitors

30

1) Principle

The assays of the bioavailability of type 3 3α -hydroxysteroid dehydrogenase inhibitors were performed in male Sprague Dawley rats

5 by measuring the plasma concentrations of the compounds after single oral administration of the compounds. The measurements at various time intervals were for values greater than or equal to 1.0 ng/mL and less than or equal to 50 ng/mL.

10 a) Animals and treatment

Male Sprague-Dawley rats [CrI:CD(SD)Br] weighing 275-350 g were obtained from Charles-River Canada Inc. and housed 2 per cage during the acclimation period and individually during the study period. The animals were maintained under a regimen of 12 hours light: 12 hours dark (lights on at 08:00). Animals received certified Rodent feed (Lab Diet # 5002, pellets) and tap water *ad libitum*. Rats were fasted (access to water only) starting on the evening prior to dosing.

Each compound to be tested was administered to three animals as a suspension in 0.4% methylcellulose by oral gavage at a dose of 0.5 mg/rat (1.0 ml/rat). Four to eight new compounds were tested each day and one group of animals received megestrol acetate (MGA) under the same conditions on each dosing day as a reference. One blood sample of ~0.7 ml was collected from the jugular vein of rats under Isoflurane-induced anesthesia at 1, 2, 3, 4, and 7 hours post-gavage. Blood samples were immediately transferred into a refrigerated 0.75 ml Microtainer containing EDTA and kept in an ice-water bath until centrifugation at 3000 rpm for 10 minutes. Plasma separation was performed rapidly (less than 50 minutes) after blood collection. One aliquot of 0.25 ml of plasma was then transferred into a borosilicate tube (13 x 100) and was rapidly frozen on dry-ice. Plasma samples were kept

5 at -80°C until measurement of plasma concentration of the inhibitor(s) by LCMS/MS.

2) LCMS measurements

a) *Apparatus*

- 10
1. Vacuum manifold
 2. Turbo Vap LV evaporator
 3. Mass spectrometer API III or API-300 (PE/Sciex) with associated peripherals
 4. Automatic Injector
 - 15 5. HPLC pump
 6. Infusion pump
 7. Calibrated pipets

b) *Reagents and Solutions*

- 20
1. Methanol, HPLC grade
 2. Water, Ultrapure (Super Q)
 3. Ethanol, reagent grade
 4. N-butyl chloride, HPLC grade
 5. Acetone, HPLC grade
 - 25 6. Male rat plasma (EDTA)
 7. type 3 3 α -hydroxysteroid dehydrogenase inhibitors in reference standard ethanol solution approximately 100 μ g/mL
 8. EM 248 Internal Standard reference standard (solution of 50 ng/mL)
 - 30 9. Mass calibrator solution Polypropylene Glycol (PE/Sciex)

- 5 c) **Mass Spectrometer Conditions**
Detector: Mass spectrometer API-300 (PE/Sciex)
Interface: Turbo Ion spray inlet (split 1/5)
Auxiliary flow: 4.5L/minute (nitrogen)
Nebulizer Flow: 11
10 Curtain Gas Flow: 11
Probe Temperature: 460 °C
Pressure: Approximately 3×10^{-5} Torr
CAD gas thickness: 3
Count Control: 1
15 Mobile Phase: Gradient of Methanol with 1 mM Ammonium
formate and Water with 1 mM Ammonium formate
Flow Rate: 1 mL/minute
- 20 d) **Mass Spectrometer Analysis Parameters for EM-1118**
Dwell time: 150 msec
Pause time: 30 msec
Duration: 4 minute
MRM mode for
EM-1118 analysis : 444.2 and 398.3
25 Injection: 10 µL
Data handling: "API Standard Software" update version.
- 30 e) **Preparation of Standard Solutions**
Stock solutions for each type 3 3α-hydroxysteroid
dehydrogenase inhibitors were prepared in methanol and,
when not in use, the methanol solutions were stored at -20°C.

5 Calibration curve standard solutions for each compound were prepared in male rat plasma as illustrated in Table 1.

10 A solution of internal standard in methanol containing EM-248 at 50 ng/mL, was prepared from stock standard solutions of EM-248 stored at -20°C.

Concentration of inhibitor 3 α -HSD	Volume of solution	Volume of plasma
Std 50 ng/mL	90 μ l of 1 μ g/mL	1.71 mL
Std 20 ng/mL	0.8 mL of 50 ng/mL	1.2 mL
Std 10 ng/mL	0.9 mL of 20 ng/mL	0.9 mL
Std 5 ng/mL	0.8 mL of 10 ng/mL	0.8 mL
Std 2 ng/mL	0.6 mL of 5 ng/mL	0.9 mL
Std 1 ng/mL	0.5 mL of 2 ng/mL	0.5 mL
Std 0	N/A	0.5 mL
Blank	N/A	0.5 mL

f) *Extraction Procedure for Type 3 Inhibitors From Rat Plasma*

15 Aliquots of rat plasma (0.250 mL) were transferred to 13 x 100 mm borosilicate tubes. Water (1.0 mL) and internal standard solution (0.1 mL) were added to each sample and vortexed for 2 min. A mixture of N-butyl chloride and acetone (v:v, 7:3) (3 mL) was added to each sample and vortexed for 2 min. This step was repeated and the combined organic phases were evaporated to dryness under nitrogen in a Turbo Vap evaporator at 35° C. The residue was reconstituted with 1 mL

20

5 of methanol and evaporated in a Turbo Vap evaporator at 35°C. The final extract was reconstituted into 0.1 mL of methanol/water (v:v, 75:25) and then transferred into a conical vial for injection into the mass spectrometer.

10 g) *Assay*

The assay procedure was performed by analyzing, in duplicate, rat plasma samples spiked at six different Type 5 inhibitor concentrations (1, 2, 5, 10, 20 and 50 ng/mL). The lower limit of quantitation (LOQ) was established at 1.0 ng/mL. Values lower than 1.0 ng/mL were expressed as below limit of quantification (BLQ).

h) *Linearity*

20 The assay procedures for EM-1118 were found to be linear over the 1.0 to 50 ng/mL range. Weighted (1/X) linear regression analysis gave a correlation (r^2) of 0.991.

i) *Calculation of AUC Values*

25 For all compounds studied, the area under the plasma concentration versus time curve (AUC) from time 0 to 7 hours post-dosing was determined. AUC_{0-7} values were calculated by the linear trapezoidal method (model-independent) for each rat and data were expressed as mean $AUC_{0-7} \pm SEM$ ($n=3$).

5 II. Enzymatic assay for types 1, 2, 3 and 5 17 β -HSD and types 1 and 3 3 α -HSD

10 Enzyme sources. 293 cells transiently transfected with expression vectors encoding types 1, 2 and 3 17 β -HSD (Luu-The et al., J. Steroid Biochem. Molec. Biol., 55: 581-587, 1995) type 5 17 β -HSD (described in WO 97/11162), and types 1 and 3 3 α -HSD (Dufort et al. Biochem. Biophys. Res. Commun. 228: 474-479, 1996), using the calcium phosphate procedure (Kingston et al., In: Current Protocols in Molecular Biology. Edited by E.M. Ausbel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, K. Struhl. John Wiley & Sons, New York, pp. 9.1.1-9.1.9, 1991; Luu-The et al., J. Invest. Dermatol., 102: 221-226, 1994). For
15 assays using cell subfractions, cells were sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA and centrifuged at 10 000 xg for 30 min before centrifugation for 100 000 x g for 1 h to separate the mitochondrial and microsomal fractions, respectively. The cytosol fractions (100 000 x g supernatant) was used to
20 determine type 1 activity while the microsomal fraction (pellet at 100 000 x g) was used for measurement of types 2 and 3 17 β -HSD activities.

25 Incubation. The enzymatic reaction was carried out at 37°C in 1 ml of 50 mM sodium phosphate buffer, pH 7.4, containing 20% glycerol, 1 mM EDTA, and 2 mM cofactors (NADPH or NAD⁺) for 1 h in the presence of 0.1 μ M ¹⁴C-labeled substrate: estrone for types 1 17 β -HSD, DHEA and 4-androstene-3,17-dione (Δ 4), for type 3 and 5 17 β -HSD, testosterone for type 2 17 β -HSD as well as androstenedione and DHT for types 1 and 3
30 3 α -HSD activities, in absence or presence of increasing concentration of preferred inhibitor of the invention, was added to freshly changed culture medium in a 6-well culture plate.

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15

After incubation for 1 h, the steroids were extracted twice with 2 ml of ether. The organic phase were pooled and evaporated to dryness. The steroids were solubilized in 50 µl of dichloromethane, applied to Silica gel 60 thin layer chromatography (TLC) plate (Merck, Darmstad, Germany) then separated by migration in the toluene-acetone (4:1) solvent system. Substrates and metabolites were identified by comparison with reference steroids and revealed by autoradiography and quantitated using the Phosphoimager System (Molecular Dynamics, Sunnyval, CA). Transfection could be also performed with HeLa, SW-13, 293, COS-1 cells, the preferred cell line is 293 cells.

III- Shionogi Activity

Androgenic/antiandrogenic activity of some preferred compounds has been measured using the Shionogi mouse mammary carcinoma cells.

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Materials. Minimal essential culture medium (MEM), non-essential amino acids, and fetal calf serum were purchased from Flow Laboratories. In order to remove endogenous steroids, serum was incubated overnight at 4 °C with 1% activated charcoal (Norit A, Fisher) and 0.1% Dextran T-70 (Pharmacia). A 2-h supplementary adsorption was performed at 25°C in order to further remove protein-bound steroids. Serum was also inactivated by a 20-min incubation at 56°C. 5 α -dihydrotestosterone (DHT) was obtained from Steraloids. The antiandrogen hydroxyflutamide (OH-FLU) was kindly supplied by Drs. T.L. Nagabuschan and R. Neri (Schering Corporation, Kenilworth, U.S.A.).

5 Cell dispersion, culture and cloning. Shionogi male mice bearing
androgen-sensitive mammary tumors were obtained from Drs. Keishi
Matsumoto, Osaka, Japan, and Yvonne Lefebvre, Ottawa, Canada. For
primary culture, tumors were excised and washed in ice-cold sterile 25
mM Hepes buffer (137 mM NaCl; 5 mM KCl; 0.7 mM Na₂HPO₄; 10 mM
10 glucose, pH 7.2). After mincing with scissors, the tumor minces were
digested for 2 h at 37°C in Hepes buffer containing 3.8 mg/ml
collagenase (Clostridium, Boehringer), 1.5 mg/ml hyaluronidase II
(Sigma), and 3% bovine serum albumin fraction V (Schwartz-Mann).
Dispersed cells were collected by centrifugation (500 x g for 10 min),
15 washed twice by suspension in minimal essential medium (MEM)
containing 5% dextran-coated charcoal-treated fetal calf serum
(DCC-FCS), 1% non-essential amino acids, 10 IU/ml penicillin, 50 µg/ml
streptomycin, and 100 nM dihydrotestosterone (DHT) (Steraloids).

20 Cells were plated in the same medium at a density of 75 000 cells/ml in
75 cm² flasks under an atmosphere of 5% carbon dioxide in air at 37°C.
The medium was changed weekly. The steroids and antisteroids were
dissolved in ethanol and kept in stock solutions chosen to yield final
ethanol concentrations less than 0.01% in the culture medium. Such a
25 concentration of ethanol does not affect cell growth.

Cells were subcultured at near-confidence by gentle digestion in a
solution of 0.1% pancreatin (Flow Laboratories) in Hepes buffer
containing 3 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.2). Cells
30 were pelleted by centrifugation, resuspended in culture medium,
counted in a Coulter counter, and replated as described above. Soft agar

5 cloning was performed as described (Stanley et al., Cell 10: 35-44, 1977) in the presence of 100 nM DHT.

Measurement of Cell Growth and Sensitivity to Steroids and Antisteroids. Cells were plated in 24-well plates at a density of 20 000
10 cells/well. The indicated increasing concentrations of agents were added to triplicate dishes, and cells were grown for 10-12 days with changes of medium every 3-4 days. Cell number was measured by direct counting in a Coulter counter.

15 Calculations and Statistical Analysis. ED₅₀ values of action of DHT and glucocorticoids were calculated according to a least-square regression as described (Rodbard, Endocrinology 94: 1427-1431, 1974). Statistical significance was calculated according to a multiple-range test (Kramer, Biometrics 12: 307-310, 1956).

20

IV- Estrogenic/Antiestrogenic Activity

Estrogenic/antiestrogenic activity of some preferred compounds has been measured using the ZR-71-1 human breast cancer cell line as described in more detail below.

25

Maintenance of Stock Cultures. ZR-75-1 cells (83rd passage) were obtained from the American Type Culture Collection (Rockville, MD) and routinely cultured in phenol red free RPMI 1640 supplemented with 1 nM estradiol (E₂), 2 mM L glutamine, 1 mM sodium pyruvate, 15 mM
30 N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid, 100 IU penicillin/ml, 100 µg streptomycin/ml, and 10% (v/v) fetal bovine serum (Hyclone, Logan, UT) under a humidified atmosphere of 95% air,

5 5% CO₂, at 37 °C. All media and medium supplements were purchased from Sigma. Cells were subcultured weekly by treatment with a pancreatic solution containing EDTA (0.2 g/L). The cell cultures used for the experiments herein described were between passages 89 and 94.

10 Measurements of Cell Proliferation. Cells in their logarithmic growth phase were harvested, briefly centrifuged, and resuspended in RPMI 1640. Cells were then plated in triplicate in LIMBRO 24-well plastic culture plates (2 cm²/well). Since plating density influences the effect of hormones on ZR-75-1 cell growth, cells were plated at a density of 1×10^4
15 cells/well. After 72 h, medium was replaced with fresh medium containing the inhibitor at the concentration of $3 \cdot 10^{-7}$ and 10^{-6} M in absence or presence of 0.1 M estradiol (E₂). Control cultures received the ethanol vehicle only. Cells were then allowed to grow at 37 °C for 10 days with medium changes (of identical composition) every 2 days. In
20 absence of inhibitors, in 0.1M estradiol (E₂)-containing medium, ZR-75-1 cells have doubling time of about 48 h.

After E₂ and/or antiestrogen treatment, cells were harvested by addition of 0.5 ml of a pancreatin solution (Sigma) for 5-10 min at 37 °C before
25 addition of 0.5 ml of RPMI 1640 containing 5% dextran coated charcoal-free bovine serum in order to block enzymatic action. Cell number (0.10 ml aliquot) was determined by measurement of DNA content as previously described (Simard et al., Endocrinology 126: 3223-3231, 1990).

5 V- Androgen Receptor (AR) Assays

Tissue Preparation. Male Sprague-Dawley rats (CrI: CD(SD)Br) weighing
200-300g were obtained from Charles-River Inc. (St-Constant, Québec,
Canada). The rats were gonadectomized under general anesthesia
(Isoflurane) and killed by cervical dislocation 24 hours later. The ventral
10 prostates were rapidly removed, dissected free from adhering tissue and
frozen on dry-ice. Prostates were kept at -80°C until assay.

 All subsequent steps were performed at 0-4°C. Prostates were
homogenized in 5 vol (wt/vol) of buffer A (25 mM Tris-HCl, 1.5 mM
15 EDTA disodium salt, 10 mM α -monothioglycerol, 10% glycerol, and 10
mM sodium molybdate, pH 7.4), using a Polytron PT-10 homogenizer
(Brinkman Instruments, Canada) at a setting of 5 for three periods of 10
sec, with intervals of 10 sec for cooling. The homogenate was then
centrifuged at 105,000 \times g for 60 min in a Beckman L5-65 ultracentrifuge
20 (Fullerton, CA). The protein concentration of the cytosol fraction was
measured according to the method of Bradford (Anal. Biochem. 72:
248-254, 1976), using bovine serum albumin as standard.

Androgen Receptor Assay. Androgen binding was measured using the
25 hydroxylapatite assay (HAP). In brief, the radioactive steroid [³H]R1881
solubilized in ethanol was diluted into buffer A. Aliquots of prostate
cytosol preparation (0.1 ml) were then incubated with 8 nM [³H]R1881
(0.1 ml, ~200,000 cpm) in the presence or absence of the indicated
concentrations of unlabeled compounds (0.1 ml, prepared in buffer A
30 containing 10% ethanol) for 16-18 h at 0-4°C. Triamcinolone acetonide
(150 nM) was added in order to mask progesterone receptors. Unbound
steroids were separated by incubation for 40 min at 0-4°C with 0.3 ml

5 HAP prepared in buffer P (50 mM, Tris-HCl, 10 mM KH_2PO_4 , pH 7.4) as follows: 10 g HAP were washed with buffer P until the supernatant reached a pH of 7.4 and then following centrifugation and decantation of the supernatant, 37.5 ml of buffer P were added. After incubation with HAP and 10 minutes of centrifugation at 1,000 x g, the pellet was washed
10 3 times with 1 ml buffer P. Thereafter, the radioactivity was extracted from the pellet by incubation at room temperature for 60 minutes with 1 ml EtOH. After centrifugation, the supernatant was decanted into a scintillation vial and the pellet was extracted again with ethanol. Thereafter, 10 ml Formula-989 scintillation liquid was added to pooled
15 supernatant and the radioactivity was measured in a Beckman counter.

Calculations. The results were reported as the percentage of inhibition of the binding of [^3H]R1881 at the concentrations of 10^{-8} and 10^{-6} M of the inhibitor.

20

VI- Progesterone Receptor Assay

Chemicals. [17α -methyl- ^3H]-promegestone (R5020) (84 Ci/mmol) and the corresponding unlabeled compound were purchased from New England Nuclear (Lachine, Québec, Canada) . All other chemicals were of
25 analytical grade.

Stock solutions of the unlabeled steroids were kept at 4°C in ethanol. The desired steroid solutions were then prepared by appropriate dilution in buffer B (10 mM Tris-HCl, 1.5 mM EDTA, 10 mM α -monothioglycerol, pH 7.4) containing 30% ethanol.
30

5 Tissue preparation. Female Sprague-Dawley rats weighing 200-300g were obtained from Charles-River Inc. (St-Constant, Québec, Canada). The rats were gonadectomized under general anesthesia (Isoflurane) and killed by cervical dislocation 24 hours later. The uteri are rapidly removed, dissected free from adhering tissue and frozen on dry-ice.
10 Tissues were kept at -80°C until use.

Cytosol preparation. All procedures were performed at 4°C. Tissues were pulverized frozen in dry ice with a Thermovac pulverizer. The samples were then homogenized in 10 vol (w/v) of buffer A (25 mM
15 Tris-HCl, 1.5 mM EDTA, 10 mM α -monothioglycerol, 10% glycerol, 10 mM sodium molybdate, pH 7.4) using a Polytron PT-10 homogenizer (Brinkmann Instruments, Canada) at a setting of 5 for two periods of 10 sec, with intervals of 10 sec for cooling. The homogenate was then centrifuged at 105,000 x g for 90 min. The supernatant was used
20 immediately for assay.

Binding Assays. Progesterone binding was measured using the dextran-coated charcoal adsorption technique. Incubations were performed at 0-4°C. for 16-18 h using 100 μ l of cytosol, 100 μ l of
25 [³H]-R5020 (5 nM final, which contained 1,000 nM of dexamethasone in order to mask the glucocorticoid receptors) and 100 μ l of unlabeled compounds at the indicated concentrations. Each concentration was done in triplicate. Assay was ended with 300 μ l of DCC (1% Norit A and 0.1% Dextran T-70 in Buffer B). After 10 min of incubation, tubes were
30 centrifuged at 2,000 x g for 10 min. and decanted in vials with 6 ml of BCS liquid scintillation (New England Nuclear, Dupont). The

5 radioactivity was measured in a Beckman counter at a counting efficiency of 35%.

Calculations. The results were reported as the percentage of the inhibition of the binding of [^3H]R5020 at the concentrations of 10^{-8} and
10 10^{-6}M of the inhibitors.

VII- Glucocorticoid Receptor Assay

Chemicals. [6,7- ^3H (N)]-Dexamethasone (39 Ci/mmol) was purchased from New England Nuclear (Lachine, Québec, Canada) while unlabeled dexametasone was obtained from Steraloids (Wilton, NH). All other
15 chemicals were of analytical grade.

Stock solutions of the unlabeled steroids were kept at 4°C in ethanol. The desired steroid solutions were then prepared by appropriate dilution in
20 buffer B (10 mM Tris-HCl, 1.5 mM EDTA, 10 mM α -monothioglycerol, pH 7.4) containing 30% ethanol.

Tissue preparation. Male Sprague-Dawley rats weighing 200-300g were obtained from Charles-River Inc. (St-Constant, Québec, Canada). The rats
25 were killed by cervical dislocation and the liver were rapidly removed, dissected free from adhering tissue and frozen on dry-ice. Tissues were kept at -80°C until use.

Cytosol preparation. All procedures were performed at 4°C . Tissues
30 were eminced and homogenized in 10 vol (w/v) of buffer A (25 mM Tris-HCl, 1.5 mM EDTA, 10 mM α -monothioglycerol, 10% glycerol, 10 mM sodium molybdate, pH 7.4) using a Polytron PT-10 homogenizer

- 5 (Brinkmann Instruments, Canada) at a setting of 5 for two periods of 10 sec, with intervals of 10 sec for cooling. The homogenate was then centrifuged at 105,000 x g for 90 min. The supernatant was used immediately for assay.

5 Binding Assays. Glucocorticoid binding was measured using the
dextran-coated charcoal adsorption technique. Incubations were
performed at 0-4°C. for 16-18 h using 100 µl of cytosol, 100 µl of
[³H]-Dexamethasone (5 nM final) and 100 µl of unlabeled compounds at
the indicated concentrations. Each concentration was done in triplicate.
10 Assay was ended with 300 µl of DCC (2.5% Norit A and 0.25% Dextran
T-70 in Buffer B). After 10 min of incubation, tubes were centrifuged at
2,000 × g for 10 min. and decanted in vials with 6 ml of BCS liquid
scintillation (New England Nuclear, Dupont). The radioactivity was
measured in a Beckman counter at a counting efficiency of 35%.

15

Calculations. The results were reported as the percentage of the
inhibition of the binding of [³H]-dexamethasone at the concentrations of
10⁻⁸ and 10⁻⁶M of the inhibitor.

20 VIII-Estrogen Receptor (ER) Assay

Tissue Preparation. Female Sprague-Dawley rats (CrI: CD(SD)Br)
weighing 200-300g were obtained from Charles-River Inc. (St-Constant,
Québec, Canada). The rats were gonadectomized under general
anesthesia (Isoflurane) and killed by cervical dislocation 24 hours later.
25 The uteri were rapidly removed, dissected free from adhering tissue and
frozen on dry-ice. Uteri were kept at -80°C until assay.

All subsequent steps were performed at 0-4°C. Uteri were homogenized
in 10 vol (wt/vol) of buffer A (25 mM Tris-HCl, 1.5 mM EDTA disodium
30 salt, 10 mM α-monothioglycerol, 10% glycerol, and 10 mM sodium
molybdate, pH 7.4), using a Polytron PT-10 homogenizer (Brinkman
Instruments, Canada) at a setting of 5 for three periods of 10 sec, with

5 intervals of 10 sec for cooling. The homogenate was then centrifuged at 105,000 x g for 60 min in a Beckman L5-65 ultracentrifuge (Fullerton, CA). The protein concentration of the cytosol fraction was measured according to the method of Bradford (Anal. Biochem. 72: 248-254, 1976), using bovine serum albumin as standard.

10 Estrogen binding was measured using the dextran-coated charcoal adsorption technique as described previously (Asselin et al., Endocrinology, 101: 666-671, 1977; Asselin and Labrie, J. Steroid Biochem., 9: 1079-1082, 1978). Briefly, [³H]E₂ solubilized in ethanol were
15 diluted into buffer A. Aliquots of uterine cytosol preparation (0.1 ml) were incubated with 5 nM [³H]E₂ (~200,000 cpm, 0.1 ml) in the presence or absence of the indicated concentrations of unlabeled compounds (0.1 ml, prepared in buffer A containing 10% ethanol) for 3 h at room temperature. Unbound steroids were then separated by incubation for 15
20 min at 0-4°C with 0.3 ml 0.5% Norit-A and 0.05% Dextran T-70 in buffer B (1.5 mM EDTA disodium salt, 10 mM monothioglycerol, and 10 mM Tris-HCl, pH 7.4) and centrifuged at 3,000 x g for 15 min. Aliquots of the supernatant (0.3 ml) were removed for radioactivity measurement. After the addition of 10 ml Formula-989 scintillation liquid (New England
25 Nuclear-DuPont), the radioactivity was measured in a Beckman counter at a counting efficiency of 62%.

Calculations. The results were reported as the percentage of inhibition of the binding of E₂ at the concentrations of 10⁻⁸ and 10⁻⁶ M of the inhibitor.

30 Primary criteria in selecting preferred inhibitors include bioavailability , desirable inhibition of type 3 3α-hydroxysteroid dehydrogenase and type

5 5 17 β -hydroxysteroid dehydrogenase, extent of undesirable inhibition on
type 2 17 β -hydroxysteroid dehydrogenase and androgenicity. It is
believed that the methyl groups in 5' position in EM 01645 and EM 01667
and analogous compounds promote selectivity of type 5 17 β -HSD
inhibition (versus undesirable type 2 inhibition). It is also believed that
10 free hydroxy group in 3-position has a benific effect as well as the
substitution in position 2.

Applicants have tested a wide range of compounds for effectiveness as
inhibitors of type 3 3 α -HSD. It is believed, based on this laboratory
15 work, that certain characteristics of molecular structure discussed herein
provide favorable characteristics to the steroidal compounds of the
invention. For example, it is believed that an aromatic A-ring and a
moiety in position 3 that is either hydroxyl or a common pro-drug group
that is converted to hydroxyl *in vivo* are characteristics which favor good
20 inhibition of type 3 3 α -HSD when combined with appropriate
substitution at position 2 or 4. Substituents at positions 2 and/or 4 are
preferably independently selected from the group consisting of hydrogen
cyano, fluoro, chloro, bromo, and nitro (provided that the substituents at
2 and 4 are not simultaneously hydrogen). The substituents that have
25 worked well at 2 position have tended to work well at 4 position and vice
versa. Placing the same substitution at both 2 and 4 may be easier from
manufacturing standpoint, and compounds of that type that we have
tested have tended to be good inhibitors of type 3 3 α -HSD.

30 Applicants have also found that inhibitors of type 3 3 α -HSD have better
selectivity when provided with D-ring substituents such as those
described herein at the 16 or 17 position of a steroidal nucleus. By

5 "selectivity", it is meant that these preferred D-ring substituents, especially those set forth at 17 position, tend to suppress undesirable interactions between the inhibitors of the invention and, for example, enzymes whose inhibition is not desired or receptors whose activation is not desired. Some of the parameters tested (both desirable and
10 undesirable) are set forth in tables herein a prime (') after their table number. (See also the detailed explanations following the tables). As may be seen from these tables, preferred compounds of the invention effectively inhibit activity of type 3 3α -HSD while substantially avoiding numerous undesirable activities for which Applicants tested the same
15 compounds. For example, appropriate D-ring substituents tend to reduce undesirable androgenic or estrogenic activities. We have found that 17-spiro-lactone and 17α -benzyl substituents give a good selectivity for type 3 3α -HSD. Not all of the type 3 3α -HSD compounds discussed herein are claimed because some of the compounds also have good
20 activity against type 5 17β -HSD and are claimed in a separate patent application by Applicants directed to this separate activity.

"In vitro" inhibition of the transformation of 4-androstenedione (4-dione) to testosterone (T) by type 3 3α -HSD.

25 The inhibition of type 3 3α -HSD was preliminarily determined using the inhibition of the transformation of DHT to androstane- $3\alpha,17\beta$ -diol as described above in "II Enzymatic assay for types 1,2,3 and 5 17β -HSD and types 1 and 3 3α -HSD" and reported in column 2 of Tables 1 and 2. To complete this data, in Tables 3 and 4, the inhibition of the transformation
30 of 4-androstenedione (4-dione) to testosterone (T) by type 3. 3α -HSD by some preferred inhibitors is reported.

5 **Enzymatic assay of the transformation of 4-androstenedione (4-dione)
to testosterone (T) by type 3 3 α -HSD**

Enzyme source:

Purified human type 3 3 α -HSD expressed over in E. coli.

10 The coding region of human type 3 3 α -HSD was amplified by PCR and
inserted in a pGEX-1 λ T(Amersham Pharmacia Biotech, Inc., Québec ,
Canada) vector in order to produce a fusion protein with glutathion-S-
transferase. Expression over of the type 3 3 α -HSD in E. coli, purification
of the protein on the glutathione-Sepharose 4B affinity column
15 (Amersham Pharmacia Biotech), and cleavage of the fusion protein by
thrombin were performed as described by the manufacturer.

Incubation:

20 The purified enzyme was incubated in a final volume of 1 ml of 50 mM
sodium phosphate buffer (pH 7.5), 20% glycerol, 1 mM EDTA and 0.1 μ M
of [14 C]-labeled steroid and 1 mM of NADPH. After 2 h incubation, the
steroids were extracted twice with 1 ml of ether. The organic phases were
pooled and evaporated to dryness. The steroids were solubilized in 50 μ l
of dichloromethane, applied to Silica gel 60 TLC plates (Merck,
25 Darmstad, Germany), before separation by migration in the toluene-
acetone (4:1) solvent system. Substrates and metabolites were identified
by comparison with reference steroids and revealed by autoradiography
and quantified using the Phosphoimager System (Molecular Dynamics,
Sunnyval, CA).

30

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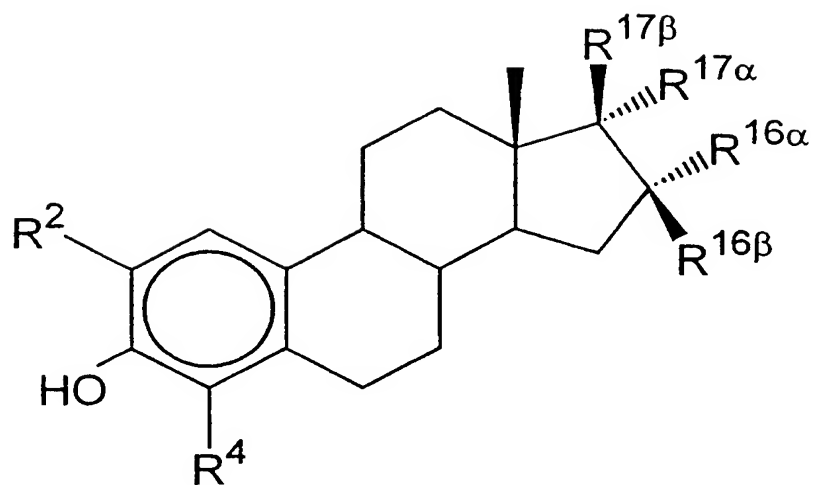
Table 3

Laboratory Name	% Inhibition of the transformation of 4-dione to T by type 3 3 α -HSD	
	E-7	E-6
EM-1124	98	99
EM-1125	72	88
EM-1126	82	95
EM-1131	80	97
EM-1667c	39	91
EM-1645	65	91

See the structures in table 1

5

Table 4

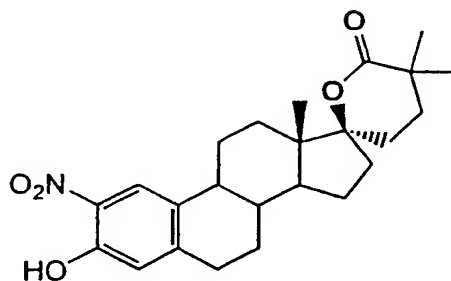


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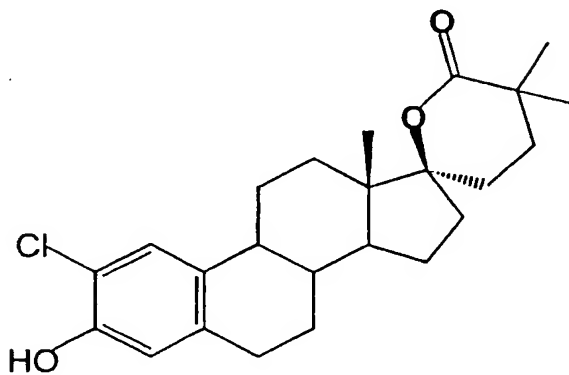
NAME	R ²	R ⁴	R ^{16α}	R ^{16β}	R ^{17α}	R ^{17β}	% Inhibition of the transformation of 4-dione to T by type 3 3α-HSD	
							E-7	E-6
EM-1926	NC-	H	CH ₃	CH ₃	H	-OH	94	97
EM-2060	NC-	H	H	H	H	-OC ₂ H ₅	88	99
EM-2200	H	-CN	H	H	=O		80	97
EM-2132	NC-	H	H	H	-C ₃ H ₇	-OH	92	99
EM-2318	NC-	H	H	H	-Ph	-OH	90	97
EM-2150	NC-	H	-CH ₂ Ph	H	=O		83	99
EM-2330	NC-	H	H	H	H	-CONH ₂	90	98
EM-2359	NC-	H	H	H	CH ₂ Φ(p-t-Bu)	-OH	79	98

- 5 Of the compounds in the foregoing tables, the most preferred and their molecular structures are set forth below :

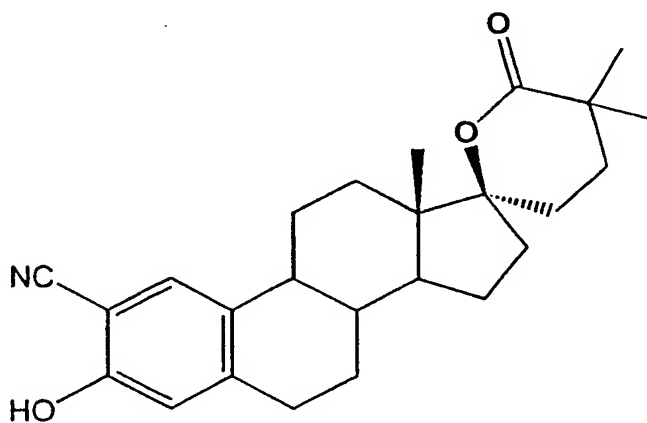
EM-1125



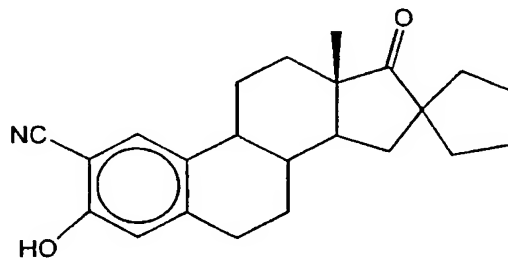
EM-01667-C



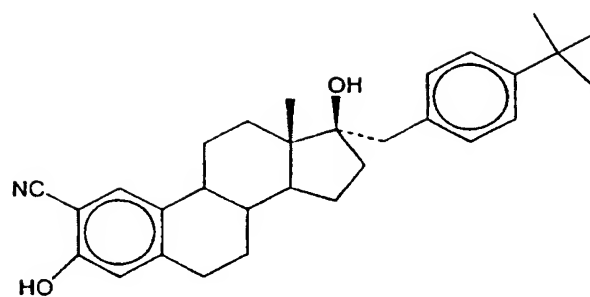
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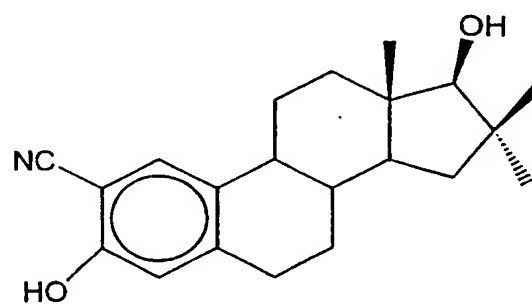
EM-1834



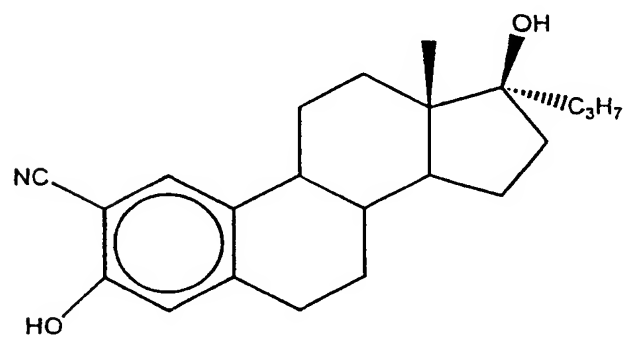
EM-2359



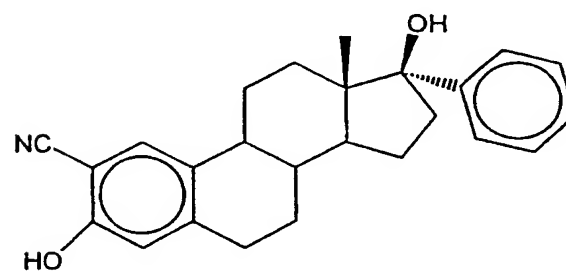
EM-1926



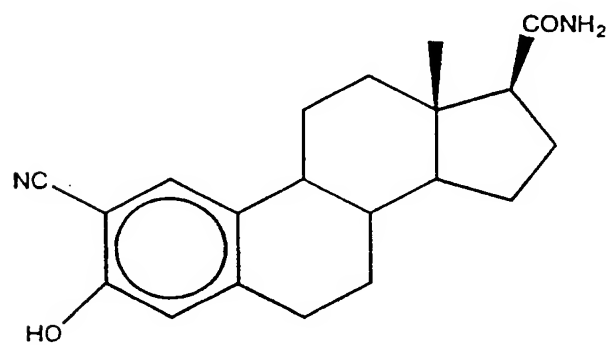
EM-2132



EM-2318



EM-2330



5 EXAMPLES OF SYNTHESIS OF PREFERRED INHIBITORS

10 The IR spectra herein were taken on a Perkin-Elmer 1600 Series FT-IR spectrophotometer. Proton NMR spectra were recorded on a Bruker AC-F 300 instrument. The following abbreviations have been used: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quadruplet; and m, multiplet. The chemical shifts (δ) were referenced to chloroform (7.26 ppm for ^1H and 77.00 ppm for ^{13}C) and were expressed in ppm. Optical rotations were measured at room temperature on a Jasco DIP 360 polarimeter. Mass spectra (MS) were obtained on a V.G. Micromass 16F machine. Thin-layer chromatography (TLC) was performed on 0.25 mm Kieselgel 60F254 plates (E. Merck, Darmstadt, FRG). For flash chromatography, Merck-Kieselgel 60 (230-400 mesh A.S.T.M.) was used. Unless otherwise noted, starting material and reactant were obtained commercially and were used as such or purified by standard means. All solvents and reactants purified and dried were stored under argon. Anhydrous reactions were performed under an inert atmosphere, the set-up assembled and cooled under argon. Organic solutions were dried over magnesium sulfate, evaporated on a rotatory evaporator and under reduced pressure. Starting materials and reagents were available from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin)

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20

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5 LIST OF ABBREVIATIONS

DHP 3,4-dihydro-2H-pyran

EDTA Ethylenediaminetetraacetic acid

HPLC High pressure liquid chromatography

PTSA p-toluenesulfonic acid

10 THF Tetrahydrofuran

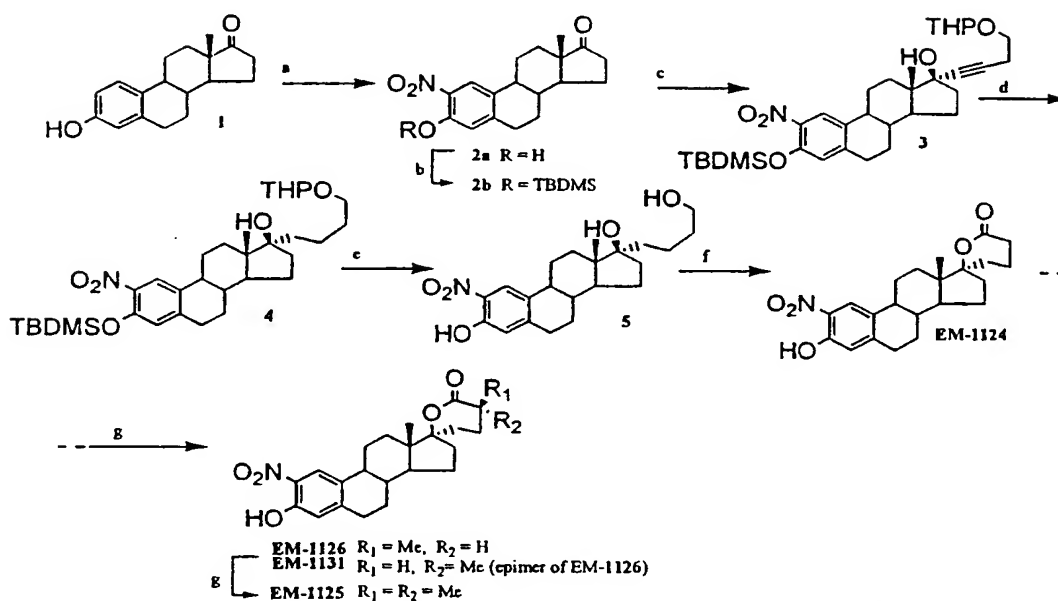
THP Tetrahydropyranyl

TMS Tetramethylsilyl

5

Example 1**Synthesis of 2-nitro-1,3,5(10)-estratrien-
17-spiro- δ -lactone derivatives**

10 These syntheses are described in Scheme 1.

Scheme 1

15

5 Example 1A

3-hydroxy-2-nitro-1,3,5(10)-estratrien-17-one (2a). The titled compound was prepared as described by Stubenrauch and Knuppen. The procedure is described below.

10 Estrone (1, 18.004 g, 66.6 mmol) was dissolved in boiling acetic acid (540 mL) and allowed to cool down to 50°C. The nitrating mixture was prepared from 70% nitric acid (4.5 mL, 70 mmol), water (10 mL) and a few crystal of sodium nitrite, warmed up to 50 °C and added dropwise to the solution of estrone with stirring. After stirring overnight at room
15 temperature, the yellow precipitate was filtered by suction and recrystallized from 92% aqueous acetic acid. 4-nitro derivative (6.800 g, 32%) was thus obtained as a pale yellow solid. IR (ν) 3227 (OH), 2931, 2864, 1723 (C=O), 1626, 1584, 1523, 1458, 1404, 1374, 1295, 1264, 1245, 1211, 1169, 1085, 1062, 1027, 954, 930, 908, 881, 823, 796, 719, 654, 588, 556,
20 530, 494 cm^{-1} ; ^1H NMR (Pyridine- d_5) δ 0.85 (3H, s, 18'-CH₃), 2.85 (2H, d, 6'-CH₂), 5.00 (1H, s, OH), 7.11 (1H, d, $J=8.7$ Hz, 2'-CH), 7.26 (1H, d, $J=8.7$ Hz, 1'-CH); ^{13}C NMR (Pyridine- d_5) δ 13.8 (C-18), 21.6 (C-15), 24.4 (C-11), 25.7 (C-7), 26.2 (C-12), 32.0 (C-6), 35.9 (C-16), 37.7 (C-8), 44.0 (C-14), 47.9 (C-13), 50.1 (C-9), 115.4 (C-2), 128.4 (C-1), 129.0 (C-10), 131.8 (C-5), 148.4
25 (C-3), 219.2 (C-17).

The reaction filtrate from above was evaporated under reduced pressure and the residue was recrystallized from EtOH/H₂O 8.5:1.5. A brown solid (7.854 g) was obtained which was further purified by flash
30 chromatography on SiO₂ column (EtOAc/hexanes, gradient 8-20%) to give pure compound 2a (6.284 g, 30%) as a yellow solid. IR (ν): 3300 (OH), 2933, 2864, 1737 (C=O), 1630, 1562, 1522, 1480, 1431, 1372, 1311,

5 1252, 1216, 1146, 1084, 1054, 1035, 1008, 905, 832, 762, 722, 662, 600, 520
cm⁻¹. ¹H NMR (Pyridine-d₅) δ 0.85 (3H, s, 18'-CH₃), 2.76 (2H, d, 6'-CH₂),
4.99 (1H, s, OH), 6.98 (1H, s, 4'-CH), 7.96 (1H, s, 1'-CH). ¹³C NMR
(Pyridine-d₅) δ 13.8 (C-18), 21.7 (C-15), 25.8 (C-11), 26.1 (C-7), 29.6 (C-12),
31.9 (C-6), 35.9 (C-16), 37.8 (C-8), 43.5 (C-14), 47.9 (C-13), 50.3 (C-9), 119.8
10 (C-4), 122.2 (C-1), 132.8 (C-10), 147.8 (C-2), 152.6 (C-3), 219.1 (C-17).

Example 1B

3-(tert-butyldimethylsilyloxy)-2-nitro-1,3,5(10)-estratrien-17-one (2b). A
15 solution of 2-nitro-estrone (2a, 1.118g, 3.55 mmole), imidazole (0.670g,
9.84 mmole) and TBDMSCl (0.781g, 5.18 mmole) in dry DMF (50 mL) was
stirred under Ar(g) overnight. The mixture was then poured onto
ice/water (80) mL. The white precipitate was filtered, washed with
water and then dried in vacuo to give (2b) as a yellowish powder (1.447
20 g, 95%). [α]_D²⁵ +123.9° (c 1.03, CHCl₃); IR (NaCl) 2933, 2860, 1736 (s, C=O),
1617, 1561, 1518, 1492, 1408, 1351, 1291, 1256, 1054, 909, 832, 790, 697 cm⁻¹;
¹H NMR δ 0.24 (6H, s, Si(CH₃)₂), 0.92 (3H, s, 18-CH₃), 1.01 (9H, s,
SiC(CH₃)₃), 1.40-1.78 (6H, m), 1.90-2.35 (5H, m), 2.37-2.60 (2H, m), 2.90
(2H, m, 6-CH₂), 6.67 (1H, s, 4-CH), 7.76 (1H, s, 1-CH); ¹³C NMR δ 220.2,
25 147.2, 143.8, 139.6, 133.3, 122.6, 122.1, 50.3, 47.9, 43.6, 37.8, 35.8, 31.3, 29.4,
26.1, 25.7, 25.6, 21.5, 18.2, 13.8, -4.4.

Example 1C

30 3-(tert-butyldimethylsilyloxy)-17β-hydroxy-2-nitro-17α-(4'-(2''-
tetrahydro-2''H-pyranyloxy)-butynyl)-1,3,5(10)-estratriene (3). To a
stirred solution of tetrahydro-2-(butynyloxy)-2H-pyran (1.71 mL, 10.91

5 mmole) in dry THF (75 mL) under Ar (g) at -35 °C was dropwise added (MeLi 1.4M in ether 7.80 mL, 10.92 mmole). The solution was stirred for 45 min. after which was added at -35 °C a solution of ketone 2b (1.294 g, 3.01 mmole) in dry THF (20 mL). After 75 min. ice (20 g) and saturated aqueous NaHCO₃ (70 mL) were added to the reaction mixture and the
10 aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried with magnesium sulfate, filtered and then concentrated in vacuo. The crude yellow oil was purified on SiO₂ (40 g, 2:8 EtOAc/hexanes) to give compound 3 as a yellow foam (1.617 g, 92%). [α]_D²⁵ -57.5° (c 0.72, CHCl₃); IR (NaCl) 3423 (broad, OH), 2936, 2870, 2366, 1654, 1630, 1578, 1560, 1527, 1481, 1458, 1438, 1313, 1268, 1121, 1080, 1032, 899, 869, 761, 669 cm⁻¹; ¹H NMR δ 0.23 (6H, s, Si(CH₃)₂), 0.87 (3H, s, 18-CH₃), 1.00 (9H, s, SiC(CH₃)₃), 1.20-2.35 (20H, m), 2.55 (2H, t, J=6.9Hz, CCCH₂), 2.84 (2H, m, 6-CH₂), 3.55 (2H, m, CH₂O of chain), 3.85 (2H, m, CH₂O of THP), 4.65 (1H, m, CH of THP), 6.65 (1H, s, 4-CH), 7.76 (1H, s, 1-CH); ¹³C NMR δ 147.0, 144.2, 139.5, 133.9, 122.6, 122.0, 98.8, 84.5, 83.4, 79.8, 65.8, 62.2, 49.4, 47.1, 43.2, 38.9, 32.6, 30.6, 29.6, 26.7, 26.2, 25.6, 25.4, 22.8, 20.4, 19.4, 18.2, 12.7, -4.4.

25 Example 1D

3-(tert-butyldimethylsilyloxy)-17β-hydroxy-2-nitro-17α-(4'-(2''-tetrahydro-2''H-pyranyloxy)-butyl)-1,3,5(10)-estratriene (4). A solution of compound 3 (2.00g, 3.42 mmol) and 5% Pd/CaCO₃ (400 mg) in dry MeOH (400 mL) was stirred under H₂(g) atmosphere (balloon) for 1 h.
30 The mixture was then filtered through celite and the filtrate rotary evaporated. The residue was purified on silica gel (2:8 EtOAc/hexanes) to give compound 4 as a white foamy solid (1.483 g, 74%). [α]_D²⁵ +31.3° (c

5 0.90, CHCl₃); IR (NaCl) 3458 (broad, OH), 2935, 2860, 1616, 1563, 1518
(NO₂), 1491, 1408, 1348 (NO₂), 1291, 1256, 1119, 1070, 1023, 925, 893, 832,
784, 672 cm⁻¹; ¹H NMR δ 0.23 (6H, s, Si(CH₃)₂), 0.91 (3H, s, 18-CH₃), 1.00
(9H, s, SiC(CH₃)₃), 1.20-2.38 (26H, m), (2.85 (2H, m, 6-CH₂), 3.48 (2H, m,
CH₂O of chain), 3.83 (2H, m, CH₂O of THP), 4.59 (1H, m, CH of THP),
10 6.65 (1H, s, 4-CH), 7.75 (1H, s, 1-CH); ¹³C NMR δ 146.9, 144.1, 139.4,
134.0, 122.4, 121.9, 98.9, 83.2, 67.6, 67.6, 62.4, 62.4, 49.3, 46.6, 36.3, 34.1,
31.3, 30.7, 30.3, 29.5, 26.9, 26.0, 25.5, 25.4, 23.3, 20.4, 19.6, 18.1, 14.3, -4.4.

Example 1E

15 **17α-(4'-hydroxybutyl)-3,17β-dihydroxy-2-nitro-1,3,5(10)-estratriene (5).**
A solution of compound 4 (300 mg, 0.510 mmol) in 5% HCl in MeOH (10
mL) was stirred at room temperature and under argon atmosphere for
12 h. The reaction mixture was then poured into NaHCO₃/ice and
MeOH was evaporated under reduced pressure. The aqueous phase was
20 extracted with EtOAc and the combined organic layers were washed
with brine, dried over MgSO₄, filtered and evaporated to dryness. This
gave a crude yellow foam (198 mg, 100%). Purification by flash
chromatography (column loaded with CH₂Cl₂ and then eluted with
EtOAc/CH₂Cl₂ 2:8, 4:6, 1:1, 6:4, 9:1) gave compound 82 as a yellow solid
25 (127.0 mg, 64%). R_f 0.21 (8:2 EtOAc/Hexanes); M.p. 184-6 °C; [α]_D²⁶
+58.8° (c. 1.00, CHCl₃); IR (ν) 3335, 2934, 2865, 1735, 1719, 1654, 1630, 1576,
1522, 1479, 1434, 1373, 1305, 1266, 1169, 1112, 1067, 1033, 1000, 896, 874,
762, 659 cm⁻¹; ¹H NMR δ 0.90 (3H, s), 3.69 (2H, d, J=5.7 Hz), 6.84 (1H, s),
7.98 (1H, s), 10.42 (1H, s); ¹³C NMR δ 14.3, 19.8, 23.3, 26.1, 26.8, 29.8, 31.2,
30 33.3, 34.3, 36.1, 39.0, 43.2, 46.5, 49.4, 61.7, 62.8, 83.4, 118.8, 121.4, 131.6,
133.7, 149.2, 152.8.

5 **Example 1F**

2-Nitro-1,3,5(10)-estraetriene-3-ol-17(R)-spiro-2'-(6'-oxo)tetrahydropyran (EM-1124). To a stirred solution of compound 5 (128 mg, 0.33mmole) in dry acetone (25 mL) at 0 °C was slowly added a first 1.1 equivalent of Jones' reagent (1.25 M, 0.29 mL, 0.80 mmol). The orange solution was then stirred for 0.5 h then a second equivalent was added. The dark solution was stirred for a further 0.5 h then quenched with isopropanol (green precipitate formed). the mixture was stirred for 10 min. then filtered through celite and the filtrate rotary evaporated. The residue was taken in EtOAc then washed with aq. sat. NaHCO₃, H₂O, brine, dried (MgSO₄), filtered, rotovaped. The crude solid was purified by flash chromatography on SiO₂ (3:7 EtOAc/Hexanes) to give EM-1124 (108 mg, 85%) as a yellow solid. M.p. 213 °C; [α]²⁵_D +90.0° (c 0.70, CHCl₃); IR (NaCl) 3198, 2934, 2876, 2245, 1720 (s,C=O, lactone), 1630, 1577, 1522, 1480, 1434, 1378, 1314, 1267, 1234, 1199, 1169, 1151, 1120, 1070, 1036, 1024, 992, 914, 851, 759, 732, 662, 585 cm⁻¹; ¹H NMR δ 1.02 (3H, s, 18-CH₃), 1.20-2.23 (16H, m), 2.25-2.65 (3H, m), 2.90 (2H, m, 6-CH₂), 6.85 (1H, s, 4-CH), 7.97 (1H, s, 1-CH), 10.41 (1H, s, OH phenol); ¹³C NMR δ 171.9, 152.8, 148.9, 133.3, 131.7, 121.5, 118.9, 93.0, 48.8, 47.1, 43.1, 38.4, 33.9, 31.6, 29.7, 29.4, 27.9, 26.8, 25.8, 23.4, 15.8, 14.2.

25

Example 1G

2-Nitro-1,3,5(10)-estratrien-3-ol-17(R)-spiro-2'-(5'-methyl-6'-oxo)tetrahydropyran (EM-1126, EM-1131). LDA was prepared as follows: To a stirred solution of diisopropylamine (92 μ L, 71 mg, 0.70 mmol) in dry THF (5 mL) at -78 °C under Ar(g) was added n-BuLi (1.2 M/Hexane, 580 μ L, 0.68 mmol) and the solution was then stirred at 0 °C for 25 min. then cooled down to -78 °C. A solution of EM-1124 (66 mg,

30

5 0.17 mmol) in dry THF (5 mL) was added and the resulting dark orange solution was then stirred for 30 min. Dry HMPA (2 mL) was added and after 15 min., MeI (107 μ L, 243 mg, 1.71 mmol). The solution was then stirred for a further 4 h. The reaction was quenched with aqueous saturated NH_4Cl and extracted with EtOAc. The organic phase was
10 washed with 1 M aqueous CuSO_4 (4X), H_2O , aqueous 1M Na_2SO_3 , brine, dried (MgSO_4), filtered then rotary evaporated to give a crude solid (103 mg). Purification by flash chromatography on SiO_2 (1:9 --> 2:8 EtOAc/Hexanes) provided first EM-1126 (11 mg, 16%) closely followed by EM-1131 (34 mg, 34%) both as yellow solids. EM-1126: M.p. 204-6 $^\circ\text{C}$;
15 $[\alpha]^{25}_{\text{D}} +73.4^\circ$ (c 1.67, CDCl_3); IR ν 3422 (br, OH), 2937, 2874, 1725 (vs, CO), 1630, 1577, 1525, 1479, 1458, 1432, 1378, 1311, 1269, 1249, 1205, 1188, 1150, 1118, 1088, 1071, 1007, 990, 934, 896, 760, 731, 668, 585, 495 cm^{-1} ; ^1H NMR δ 1.03 (3H, s), 1.30 (3H, d, $J=7.1$ Hz), 1.31-1.77 (10H, m), 1.89-2.03 (5H, m), 2.15 (1H, td, $J=7.1$ Hz, $J'=5.0$ Hz), 2.30-2.50 (2H, m), 2.90 (2H, dd, $J=8.3$ Hz, $J'=4.9$ Hz), 6.85, (1H, s), 7.98 (1H, s), 10.43 (1H, s, OH); ^{13}C NMR δ 174.8,
20 152.9, 149.0, 133.4, 131.7, 121.5, 118.9, 93.4, 48.7, 47.1, 43.1, 38.5, 36.2, 34.6, 31.6, 29.7, 28.6, 26.9, 25.9, 25.2, 23.4, 17.4, 14.4.

EM-1131 (5'-epimer of EM-1126, real configuration not determined):
25 M.p. 206-8 $^\circ\text{C}$; $[\alpha]^{25}_{\text{D}} +62.6^\circ$ (c 0.68, CDCl_3); IR ν 3422 (br, OH), 3192, 2934, 2876, 2858, 2824, 1721 (vs, CO), 1631, 1578, 1522, 1482, 1458, 1436, 1377, 1314, 1271, 1237, 1204, 1173, 1120, 1103, 1082, 1051, 1019, 1002, 933, 901, 877, 860, 759, 663, 638, 600, 495 cm^{-1} ; ^1H NMR δ 1.01 (3H, s), 1.24 (3H, d, $J=7.0$ Hz), 1.31-1.80 (10H, m), 1.89-2.20 (6H, m), 2.35 (1H, br s), 2.55
30 (1H, sextuplet, $J=7.5$ Hz), 2.89 (2H, t, $J=5.2$ Hz), 6.84 (1H, s), 7.96 (1H, s), 10.41 (H, s, OH); ^{13}C NMR δ 175.8, 152.8, 148.9, 133.3, 131.6, 121.4, 118.8,

5 92.5, 77.4, 77.0, 76.6, 48.5, 47.0, 43.0, 38.4, 33.8, 33.4, 31.6, 29.7, 27.16, 26.7, 25.8, 24.3, 23.6, 17.2, 14.3.

Example 1H

2-Nitro-1,3,5(10)-estratrien-3-ol-17(R)-spiro-2'-(5',5'-dimethyl-6'-
10 oxo)tetrahydropyran (EM-1125). LDA was prepared as follows: To a stirred solution of diisopropylamine (206 μ L, 159 mg, 1.57 mmol) in dry THF (12 mL) at -78 °C under Ar(g) was added n-BuLi (1.2 M/Hexane, 1.28 mL, 1.53 mmol) and the solution was then stirred at 0 °C for 20 min. then cooled down to -78 °C. A solution of a mixture of EM-1126 and EM-
15 1131 (153 mg, 0.38 mmol) in dry THF (10 mL) was added and the resulting dark orange solution was then stirred for 20 min. Dry HMPA (4.7 mL) was added and after 15 min., MeI (238 μ L, 544 mg, 3.83 mmol). The solution was then stirred for 5 min. then was warmed up to -30 °C and stirred for a further 1 h. The reaction was quenched with aqueous
20 saturated NH₄Cl and extracted with EtOAc. The organic phase was washed with brine (6X), aqueous 1M Na₂SO₃, brine, dried (MgSO₄), filtered then rotary evaporated to give a crude liquid. Purification by flash chromatography on SiO₂ (1:9 --> 2:8 EtOAc/Hexanes) provided EM-1125 (82 mg, 52%) as a yellow solid. M.p. 195-7 °C; [α]_D²⁵ +72.8 ° (c
25 1.61, CDCl₃); IR ν 3421 (br, OH), 3194, 2954, 2927, 2873, 1718 (vs, CO), 1631, 1578, 1523, 1476, 1458, 1438, 1386, 1312, 1298, 1271, 1204, 1151, 1118, 1059, 1032, 1016, 931, 898, 872, 855, 758, 663, 595 cm⁻¹; ¹H NMR δ 1.02 (3H, s), 1.28 (6H, s), 1.32-1.77 (10H, m), 1.85-2.15 (6H, m), 2.36 (1H, br s), 2.89 (2H, dd, *J*=8.2 Hz, *J'*=4.9 Hz), 6.85 (1H, s), 7.97 (1H, s), 10.42 (H, s,
30 OH); ¹³C NMR δ 177.7, 152.8, 149.0, 133.3, 131.7, 121.5, 118.9, 93.4, 48.6, 47.1, 43.1, 38.5, 37.8, 34.7, 31.6, 31.5, 29.7, 27.7(4), 27.6(8), 26.7, 25.9, 25.5, 23.3, 14.4.

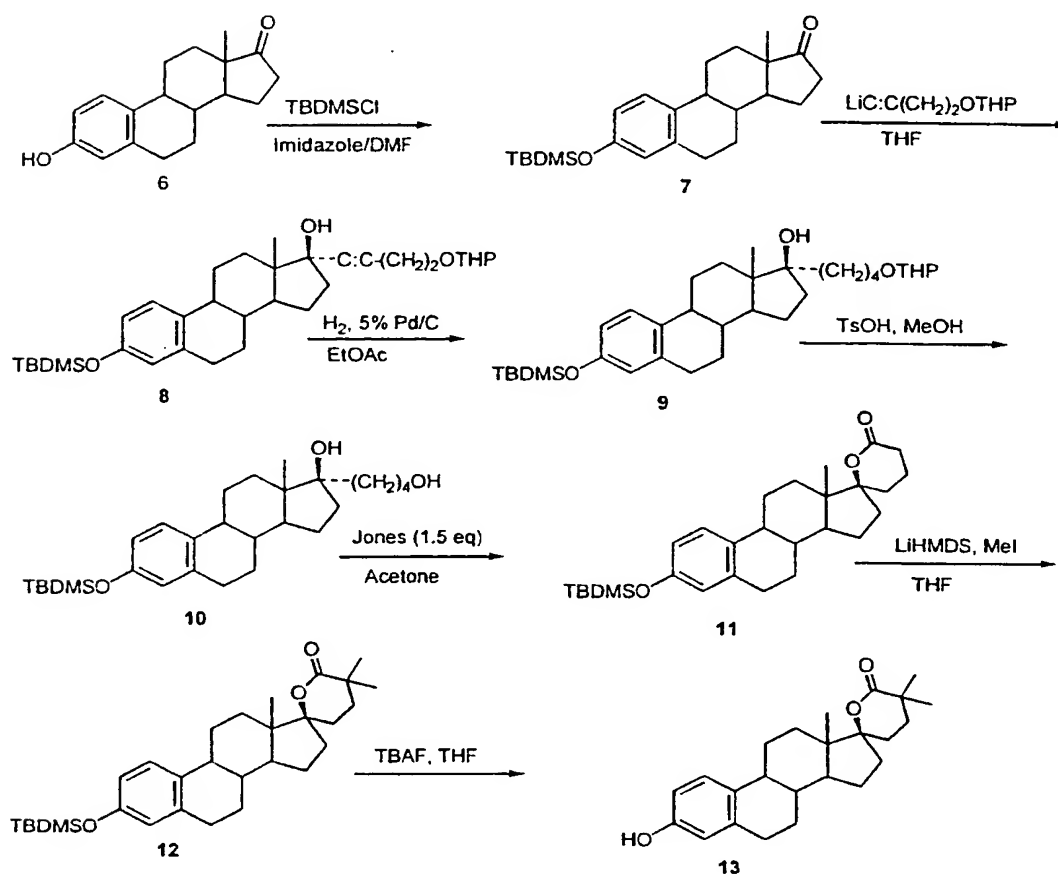
5

Example 2

Synthesis of 2-cyano-1,3,5(10)-estratrien-30'-17(R)-spiro-2'-
(5',5'-dimethyl-6'-oxo) tetrahydropyran (13)

10

Scheme 2



5 3-t-butyldimethylsilyloxy-1,3,5(10)-Estratrien-17-one (7). The ether was prepared from estrone (6) following the method described Pelletier et al. (Steroids 59: 536-547, 1994).

10 3-t-butyldimethylsilyloxy-17 β -hydroxy-17 α -[4'-(2"-tetrahydro-2"H-pyranyl)butyn-1'-yl]-1,3,5(10)-estratriene (8). To a solution of HC \equiv C(CH₂)₂OTHP (18.3 mL, 117 mmol) in dry THF (600 mL) at 0 °C, was added dropwise n-butyllithium (43.7 mL, 109 mmol) and the mixture was stirred for 90 min. The mixture was cooled to -78 °C and a solution of TBDMS-estrone 7 (15 g, 39 mmol) in THF (500 mL) was added dropwise.

15 Then, the reaction mixture was allowed to come to room temperature and left stirring for a period of 15 h. Solvents were evaporated to the half volume and 200 mL of water was added. The mixture was extracted with EtOAc (3 x 200 mL), the organic layer was washed with brine, dried (MgSO₄) and evaporated to dryness. The residue was purified over silica

20 gel column chromatography with hexanes/EtOAc (9/1) as an eluent to furnish 15.1 g (72%) of the product; IR (NaCl cm⁻¹) 3432, 2934, 2858, 1607, 1495, 1287, 1256, 1033, 958, 839; ¹H NMR (300 MHz, CDCl₃) δ 7.12 (d, 1H, J=8.4 Hz), 6.62 (dd, 1H, J=2.4, 8.4 Hz), 6.54 (d, 1H, J=2.2 Hz), 4.66 (br.s., 1H), 3.89-3.79 (m, 2H), 3.56-3.50 (m, 2H), 2.79 (br.s., 2H), 2.56 (t, 2H, J=7.0 Hz), 2.35-2.17 (m, 3H), 2.07-1.23 (m, 17H), 0.98 (s, 9H), 0.87 (s, 3H, 18-Me),

25 0.19 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 153.3, 137.8, 133.0, 126.1, 119.9, 117.1, 98.7, 84.7, 83.2, 80.0, 65.8, 62.1, 49.5, 47.2, 43.7, 39.4, 39.0, 32.9, 30.6, 29.7, 27.3, 26.4, 25.7, 25.4, 22.8, 20.3, 19.3, 18.1, 12.8, -4.4.

30 3-t-butyldimethylsilyloxy 17 β -Hydroxy-17 α -[4'-(2"-tetrahydro-2"H-pyranyl)butan-1'-yl]-1,3,5(10)-estratriene (9). 5% Palladium on activated carbon (1.5 g, 10% wt) was added to a solution of the alkyne 8 (15.1 g, 28

5 mmol) in EtOAc (500 mL) at room temperature. The flask was purged with H₂ three times (vacuum followed by H₂) and left stirring under 1 atm pressure of H₂. The reaction was followed by TLC. After a period of 3 h, the mixture was filtered over a plug of celite and the solvent was removed under reduced pressure. The crude product was used in the next step
10 without further purification; IR (NaCl, cm⁻¹) 3474, 2935, 2858, 1607, 1570, 1496, 1471, 1286, 1257, 1156, 1137, 1119, 1033, 954, 839, 780; ¹H NMR (300 MHz, CDCl₃) δ 7.12 (d, 1H, *J*=8.4 Hz), 6.62 (dd, 1H, *J*=2.1, 8.4 Hz), 6.55 (s, 1H), 4.59 (br.s., 1H), 3.92-3.73 (m, 2H), 3.55-3.38 (m, 2H), 2.82-2.77 (m, 2H), 2.30-1.33 (m, 26H), 0.97 (s, 9H), 0.90 (s, 3H, 18-Me), 0.18 (s, 6H); ¹³C NMR
15 (75 MHz, CDCl₃) δ 153.27, 137.81, 133.08, 126.02, 119.87, 117.06, (98.90, 98.84), 83.38, 67.61, 62.33, 49.50, 46.67, 43.81, 39.58, 36.35, 34.28, 31.60, 30.75, 30.36, 29.62, 27.51, 26.26, 25.67, 25.47, 23.37, 20.45, 19.66, 18.12, 14.35, -4.43.

3-*t*-butyldimethylsilyloxy 17β-hydroxy-17α-(4'-hydroxybutan-1'-yl)-
20 **1,3,5(10)-estratriene (10).** To a solution of the THP ether 9 (15.1 g, 28 mmol) in MeOH (400 mL), was added *p*-toluenesulfonic acid monohydrate (150 mg, 0.8 mmol) and the reaction was stirred over a period of 5 h. A saturated solution of NaHCO₃ (100 mL) was added and volume of solvent was reduced to half on a rotary evaporator. The mixture was extracted
25 with CH₂Cl₂, the organic phase was washed with brine, dried (MgSO₄) and evaporated to dryness. The crude product was used in the next step without purification; IR (NaCl, cm⁻¹) 3356, 2931, 2858, 1608, 1496, 1471, 1286, 1256, 954, 839, 780; ¹H NMR (300 MHz, CDCl₃) δ 7.12 (d, 1H, *J*=8.5 Hz), 6.61 (dd, 1H, *J*=2.5, 8.5 Hz), 6.55 (s, 1H), 3.69 (br.d, 2H, *J*=5.2 Hz), 2.82-
30 2.78 (m, 2H), 2.35-2.26 (m, 1H), 2.20-1.94 (m, 2H), 1.90-1.81 (m, 1H), 1.62-1.22 (m, 17H), 0.98 (s, 9H), 0.90 (s, 3H, 18-Me), 0.19 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 153.27, 137.81, 133.05, 126.02, 119.89, 117.09, 83.59, 62.56,

5 49.50, 46.69, 48.81, 39.58, 35.98, 34.32, 33.20, 31.61, 29.62, 27.51, 26.26, 25.68, 23.37, 19.74, 18.14, 14.36, -4.40.

3-*t*-butyldimethylsilyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(6'-oxo) tetrahydropyran (11). To a solution of the diol 10 (12.5 g, 27 mmol) in acetone (500 mL) at 0°C, was added dropwise of a 2.7 M solution of Jone's reagent (15.1 mL, 41 mmol). The reaction was stirred for 30 min. 2-Propanol (100 mL) was added, followed by of a saturated solution of NaHCO₃ (200 mL). The volume of solvents was reduced to half by evaporation and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried (MgSO₄) and concentrated under reduced pressure. The residue was purified a silica gel column chromatography with hexanes/acetone (6/1) to afford 8.6 g of the lactone. (68% yield for 3 steps); IR (NaCl, cm⁻¹): 2960, 2930, 2857, 1732, 1607, 1496, 1284, 1264, 1244, 1037, 958, 840; ¹H NMR (300 MHz, CDCl₃) δ 7.11 (d, 1H, *J*=8.4 Hz), 6.61 (dd, 1H, *J*=2.3, 8.4Hz), 6.56 (s, 1H), 2.85-2.79 (m, 2H), 2.58-2.39 (m, 2H), 2.38-2.25 (m, 1H), 2.21-2.10 (m, 1H), 2.03-1.27 (m, 15H), 1.02 (s, 3H, 18-Me), 0.97 (s, 9H), 0.18 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 172.00, 153.36, 137.63, 132.62, 126.02, 119.92, 117.19, 93.25, 48.88, 47.26, 43.68, 39.05, 33.98, 31.96, 29.50, 29.48, 27.94, 27.46, 25.98, 25.67, 23.48, 18.12, 15.87, 14.30, -4.43.

25 3-*t*-butyldimethylsilyloxy-1,3,5(10)-estratrien-17(R)-spiro-2'-(5'-5'-dimethyl-6'-oxo) tetrahydropyran(12). In a dry 1L flask under argon, was dissolved the lactone 11 (8.6 g, 19 mmol) in dry THF (300 mL), and cooled to 0°C. A 1M solution of LiHMDS (47.3 mL, 47.3 mmol) was added dropwise. The mixture was stirred 15 min at 0°C and cooled to -78°C and then methyl iodide (5.9 mL, 79 mmol) was added. The reaction was stirred 30 1 h at this temperature and then allowed to warm to room temperature

5 over a period of 2 h. A saturated solution of NH_4Cl (200 mL) was added and the mixture was extracted with EtOAc. The organic layer was washed with a saturated solution of $\text{Na}_2\text{S}_2\text{O}_3$, brine, dried (MgSO_4) and concentrated under reduced pressure. The residue was purified by column chromatography with hexanes/acetone (5/1) as an eluent to afford 7.4 g
10 (81%) of the dimethyl compound; IR (NaCl, cm^{-1}) 2954, 2930, 2858, 1725, 1496, 1287, 1258, 1150, 1137, 956, 840; ^1H NMR (300 MHz, CDCl_3) δ 7.11 (d, 1H, $J=8.5\text{Hz}$), 6.62 (dd, 1H, $J=2.4, 8.5\text{Hz}$), 6.55 (d, 1H, $J=2.1\text{Hz}$), 2.81-2.78 (m, 2H), 2.36-2.28 (m, 1H), 2.20-1.38 (m, 16H), 1.28 (s, 3H), 1.27 (s, 3H), 1.02 (s, 3H, 18-Me), 0.97 (s, 9H), 0.18 (s, 6H); ^{13}C NMR (75 MHz, CDCl_3) δ
15 177.79, 153.33, 137.62, 132.62, 125.99, 119.90, 117.14, 93.66, 48.67, 47.24, 43.65, 39.06, 37.74, 34.79, 31.96, 31.56, 29.50, 27.73, 27.61, 27.42, 26.01, 25.65, 25.55, 23.26, 18.11, 14.42, -4.43.

**1,3,5(10)-estratrien-3-ol-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)
20 tetrahydropyran (13):** To a solution of the silyl ether 12 (7.1 g, 14.7 mmol) in THF (300 mL) at 0°C , was added dropwise a 1M solution of TBAF (17.6 mL, 17.6 mmol) and the reaction was stirred for 15 min. Ice water (200 mL) was added to precipitate the compound. The flask was placed on a rotary evaporator to reduce the volume of THF, and then placed on an ice bath.
25 The precipitate was collected by filtration, washed with cold water and dried in an oven (30°C) over a period of 24 h to furnish 5.4 g (100%) of the 3-OH compound; IR (NaCl, cm^{-1}): 3357, 2932, 2871, 1695, 1287, 1158; ^1H NMR (300 MHz, CDCl_3) δ 7.14 (d, 1H, $J=8.4\text{Hz}$), 6.63 (dd, 1H, $J=2.6, 8.4\text{Hz}$), 6.55 (d, 1H, $J=2.6\text{Hz}$), 4.62 (br.s, 1H, OH), 2.81-2.79 (m, 2H), 2.38-2.29 (m, 1H), 2.20-1.81 (m, 5H), 1.76-1.31 (m, 11H), 1.29 (s, 3H), 1.28 (s, 3H), 1.01
30 (s, 3H, 18-Me); ^{13}C NMR (75 MHz, CDCl_3) δ 178.06, 153.52, 138.08, 132.19,

5 126.42, 115.26, 112.74, 93.80, 48.69, 47.29, 43.65, 39.14, 37.81, 34.84, 31.98,
31.61, 29.53, 27.76, 27.64, 27.39, 26.12, 25.59, 23.29, 14.43.

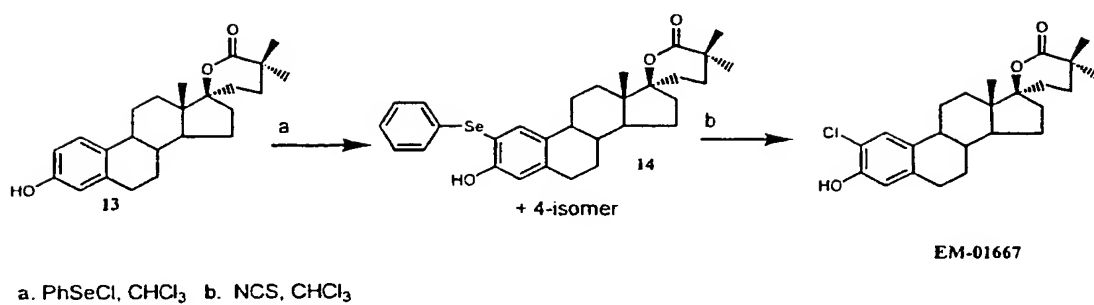
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Example 3

Synthesis of EM-01667

Scheme 3

10



5 **3-Hydroxy-2-phenylselenenyl-estra-1,3,5(10)-triene-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo) tetrahydropyran (14).** A solution of 3-hydroxy-estra-1,3,5(10)-triene-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (406 mg, 1.10 mmol) (13) and phenylselenenyl chloride (253 mg, 1.32 mmol) in dry CHCl_3 (24 mL) under Ar (g) was stirred at 0 °C for one hour then at
10 r.t. overnight. The resulting yellow solution was poured onto ice/ H_2O then extracted with CH_2Cl_2 (3x). The combined organic phase was dried (cotton plug) then rotary evaporated to give a crude foamy solid. Purification by flash chromatography (SiO_2) using 1:9 EtOAc/Hexane as eluent gave 7 (353 mg, 61%) with the 4-isomer (86 mg, 15%). Compound
15 7: $[\alpha]_D^{25} +77.7^\circ$ (c 1.14, CHCl_3); IR ν 3366, 3050, 2965, 2928, 2869, 1709, 1603, 1576, 1550, 1458, 1438, 1384, 1349, 1310, 1294, 1262, 1202, 1157, 1141, 1114, 1065, 1017, 984, 892, 845, 736, 689, 665, 593, 555, 498, 460 cm^{-1} ; ^1H NMR(CDCl_3) (δ) 1.02 (3H, s), 1.27(9) (s, 3H), 1.28(4) (s, 3H), 1.27-1.80 (11H, m), 1.88-2.28 (6H, m), 2.87 (2H, t, $J = 4.8$ Hz), 6.24 (1H, s, OH), 6.80
20 (1H, s), 7.21 (5H, br s), 7.52 (1H, s) ppm; ^{13}C NMR(CDCl_3) (δ) 14.4, 23.3, 25.5, 26.1, 27.2, 27.6, 27.7, 29.5, 31.5, 31.8, 34.7, 37.7, 38.9, 43.4, 47.2, 48.6, 93.6, 111.6, 114.7, 126.5, 129.2(6), 129.3(4), 131.2, 133.3, 134.7, 141.4, 154.4, 177.8 ppm.

25 **2-Chloro-3-hydroxy-estra-1,3,5(10)-triene-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo) tetrahydropyran (EM-01667).** A solution of 14 (116 mg, 0.22 mmol) and N-chlorosuccinimide (44 mg, 0.33 mmol) in dry CHCl_3 (15 mL) under Ar(g) at 0 °C were stirred for 30 min. The solution was poured onto ice/ H_2O then was extracted with CH_2Cl_2 (3x). The
30 combined organic phase was dried (cotton plug) then rotary evaporated to give a crude solid. Purification by flash chromatography (SiO_2) using 1:29->1:19 EtOAc/Toluene as eluent gave EM-01667 (51 mg, 57%) as a

5 white solid. Rf 0.28 (3:7 EtOAc/Hexane); m.p. 241 °C; $[\alpha]^{25}_D +62.6^\circ$ (c
1.09, CDCl_3); IR (ν) 3253 (br, OH), 2936, 2873, 1685 (CO), 1608, 1501, 1458,
1418, 1388, 1314, 1300, 1263, 1223, 1160, 1016, 987, 884, 844, 737, 673, 598
cm⁻¹; ^1H NMR (CDCl_3) (δ) 1.01 (3H, s), 1.28 (6H, s), 1.25-1.75 (11H, m),
1.85-2.28 (6H, m), 2.80 (2H, dd, $J'=8.7$ Hz, $J''=3.9$ Hz), 5.33 (1H, br s, OH),
10 6.73 (1H, s), 7.19 (1H, s) ppm; ^{13}C NMR(CDCl_3) (δ) 14.4, 23.3, 25.6, 26.1,
27.2, 27.7, 27.8, 29.0, 31.6, 31.8, 34.8, 37.8, 38.8, 43.4, 47.2, 48.6, 93.6, 116.0,
117.1, 125.6, 133.5, 137.1, 149.0, 177.8 ppm.

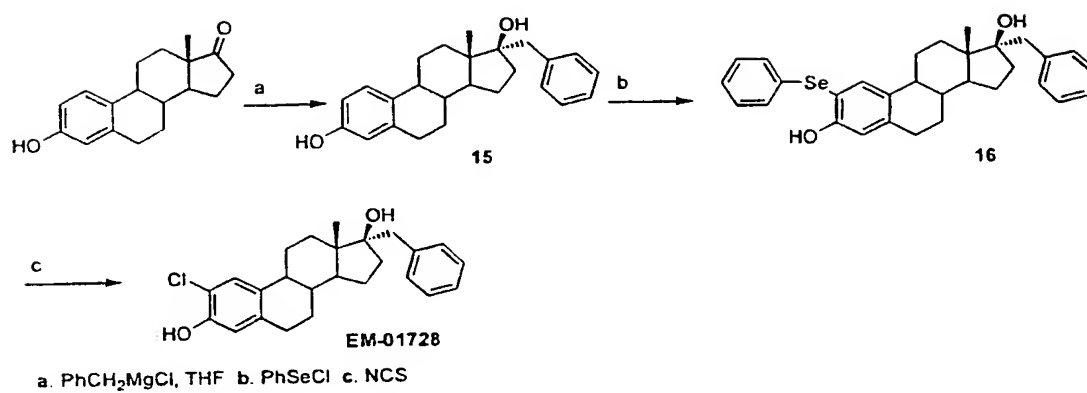
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Example 4

Synthesis of EM-01728

Scheme 4

10



5 **17-benzyl-1, 3, 5, (10)estratriene-3,17 β -diol (15)**

To a solution of estrone (5.0 g, 18.5 mmol) in dry THF (200 mL) at 0°C under Ar(g) was added benzyl magnesium chloride (2M in THF, 65 mL) and the solution was stirred overnight. Aqueous saturated NH₄Cl

10 solution was added at 0°C and the solution was extracted with CH₂Cl₂ (3 times), washed with brine, dried with MgSO₄, filtered and then evaporated. The product was purified by flash chromatography (RP-C18, 30:30:40 - 10:30:60, H₂O/MeOH/CH₃CN) to give **1** as a white solid (4.0 g, 60%) and estrone: (1.5g, 30% recovery). Compound **1**: Rf: 0.25 (2:98, MeOH/ CH₂Cl₂); IR (ν) 3284, 2926, 2856, 1725, 1686, 1655, 1606, 1561, 1499, 1439, 1378, 1343, 1321, 1286, 1252, 1221, 1156, 1082, 1029, 1016, 930, 915, 888, 872, 820, 786, 732, 700, 646, 586 cm⁻¹; ¹H NMR (CD₃OD) (δ) 0.94 (3H, s, H18), 1.32-2.35 (13H, m), 2.67 (1H, d, J=13.5 Hz, CH₂-Ph), 2.78 (2H, m, H6), 2.87 (1H, d, J=13.5 Hz, CH₂-Ph), 6.49 (1H, d, J=2.4 Hz, H4), 6.56 (1H, dd, J=8.3 Hz, J'=2.5 Hz, H2), 7.10 (1H, d, J=8.3 Hz, H1), 7.18-7.30 (5H, m, Ph) ppm; ¹³C NMR (CD₃OD) (δ) 15.3, 24.1, 27.7, 28.8, 30.8, 32.4, 32.8, 41.4, 43.6, 45.2, 50.9, 84.5, 113.7, 116.1, 126.9, 127.2, 128.7, 132.3, 136.6, 138.8, 140.3, 155.9 ppm.

25 **17-benzyl-2-Phenylselenenyl-1, 3, 5, (10)estratriene-3,17 β -diol (16)**

To a stirred solution of **1** (508 mg, 1.40 mmol) in MeOH/CHCl₃ (1:10, 33 mL) at 0°C was added PhSeCl (322 mg, 1.68 mmol). After two hours, the orange solution had turned yellow and was poured onto ice/H₂O, extracted with CH₂Cl₂, dried (MgSO₄), filtered and then evaporated. The crude solid was purified by flash chromatography (SiO₂, 1:29 EtOAc/Toluene) to give **2** as a beige solid (456 mg, 63%) and the 4-phenylselenenyl isomer (71 mg, 10%). Compound **2**: ¹H NMR (δ) 1.00 (3H, s, H18), 1.30-2.38 (14H, m), 2.82 (2H, dd, J=78.1 Hz, J'=13.2 Hz, CH₂-Ph),

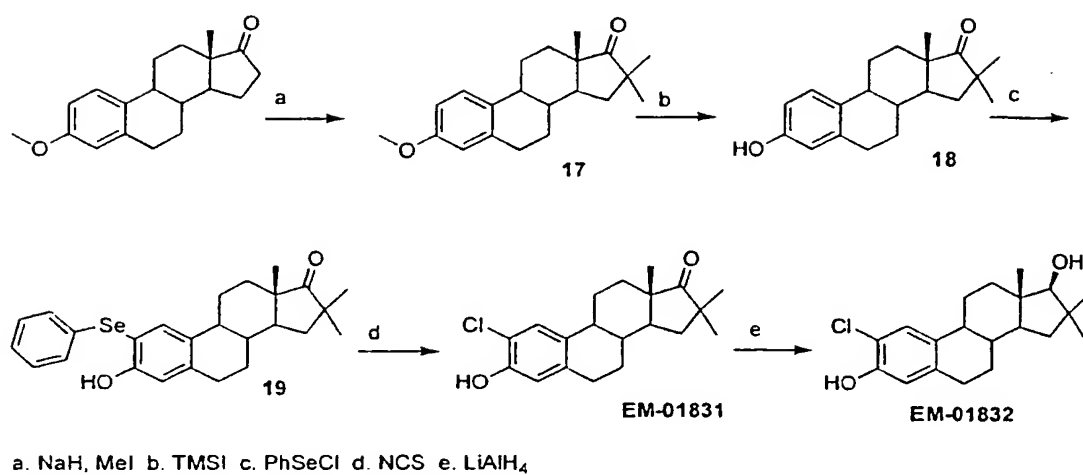
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- 5 2.90-2.93 (2H, m, H6), 6.26 (1H, br s, OH), 6.85 (1H, s, H1), 7.21-7.38 (10H, m), 7.59 (1H, s, H4), ¹³C NMR (δ) 14.5, 23.4, 26.4, 27.4, 29.7, 31.3, 33.7, 39.4, 42.4, 43.7, 46.7, 49.5, 82.9, 111.5, 114.7, 126.3, 126.5, 128.1, 129.2, 129.3, 131.0, 131.3, 133.7, 134.8, 138.2, 141.6, 154.4 ppm.
- 10 **17-Benzyl-2-chloro-1, 3, 5, (10)estratriene-3,17β-diol (EM-01728)**
To a stirred solution of 2 (155 mg, 0.30 mmol) in CHCl₃ (15 mL) at 0°C under Ar (g) was added N-chlorosuccinimide (48 mg, 0.36 mmol). After one hour, the reaction was worked up as in 2 and purification by flash chromatography (SiO₂, 1:29 → 1:19 EtOAc/Toluene) gave EM-01728 as a
- 15 white solid (74 mg, 62%). R_f 0.18 (1:19 EtOAc/Toluene); M.p. 220°C; [α]_D²⁵ +74.8° (c 0.96, acetone-d₆); IR (ν) 3544 (OH), 3284 (br, OH), 3023, 2931, 2849, 1601, 1497, 1454, 1338, 1285, 1257, 1201, 1084, 1015, 979, 918, 885, 796, 755, 702, 675, 642, 560, 532, 504 cm⁻¹; ¹H NMR (CD₃OD) (δ) 0.95 (3H, s, H18), 1.33-1.75 (15H, m), 2.68 (1H, d, J=15.5 Hz, CH₂-Ph), 2.76 (1H, dd, J=8.3 Hz, J'=3.6 Hz, H6), 2.88 (1H, d, J=15.5 Hz, CH₂-Ph), 6.60 (1H, s, H4), 7.16 (1H, s, H1), 7.17-7.31 (5H, m, Ph) ppm; ¹³C NMR (acetone-d₆) (δ) 15.2, 24.0, 27.3, 28.3, 29.9, 32.1, 33.5, 40.7, 43.5, 44.6, 48.0, 50.3, 83.6, 117.5, 118.3, 126.6, 127.5, 128.5, 132.2, 134.2, 137.8, 140.6, 151.3 ppm.
- 20

5

Example 5**Synthesis of EM-01831 and EM-01832****Scheme 5**

10



5 **16,16-Dimethyl-3-methoxy-1,3,5(10)-estratrien-17-one (17)**

To a solution of 3-methoxy-estra-1,3,5(10)triene-17-one (10.00 g, 35 mmol) in anhydrous THF (500 mL) under Ar(g) at room temperature was added NaH (60% in oil, 2.55 g, 105 mmol) and iodomethane (22 mL, 350 mmol). The solution was refluxed overnight. More NaH (2.55 g, 105 mmol) and
10 iodomethane (22 mL, 350 mol) was then added and the solution was refluxed for another 24 hours. The resulting solution was quenched with ethanol (100 mL) and then by water onto ice (300 mL). This solution was extracted using ethyl acetate (2 x 300 mL), washed with brine (2 x 300 mL), dried with MgSO₄ and evaporated under reduce pressure to give a
15 yellow solid. Purification by flash chromatography on silica gel using ethyl acetate/hexane (1:19) as eluent gave 3 (10.19 g, 93%) as a white solid. IR (ν) 2933, 2869, 1731 (CO), 1609, 1502, 1467, 1382, 1315, 1258, 1240, 1153, 1037, 1020, 902, 850, 816, 781 cm⁻¹; ¹H NMR (δ) 0.94 (3H, s, H18), 1.09 (3H, s, 16-Me), 1.22 (3H, s, 16-Me), 1.40-2.42 (11H, m), 2.90 (2H, dd, J=8.0 Hz, J'=3.3 Hz, H6), 3.79 (3H, s, OMe), 6.65 (1H, d, J=2.7 Hz, H4),
20 6.73 (1H, dd, J=8.4 Hz, J'=2.7 Hz, H2), 7.21 (1H, d, J=8.5 Hz, H1) ppm; ¹³C NMR (δ) 14.4, 25.8, 26.0, 26.7, 27.3, 29.7, 32.3, 37.6, 37.9, 44.2, 45.3, 47.2, 49.0, 55.2, 111.5, 113.9, 126.3, 132.2, 137.7, 157.6, 225.1 ppm.

25 **16,16-Dimethyl-1,3,5(10)-estratrien-3-ol-17-one (18)**

A solution of 3-methoxy-estra-1,3,5(10)triene-17-one-16-dimethyl, 17 (6.00 g, 19 mmol) and iodotrimethylsilane (27 mL, 190 mmol) in anhydrous CH₂Cl₂ (1000 mL) under Ar(g) was refluxed overnight. The resulting solution was poured onto ice/H₂O (600 mL), extracted with CH₂Cl₂ (3 x
30 600 mL), dried with MgSO₄, filtered and then evaporated under reduce pressure. The crude brown solid was purified by flash chromatography on silica gel using ethyl acetate/toluene (1:9) as eluent to give 4 as a

5 white solid (3.50 g, 62%). IR (ν) 3361 (br,s, OH), 3027, 2923, 2876, 1860 (w), 1717 (CO), 1620, 1584, 1499, 1460, 1355, 1286, 1248, 1152, 1099, 1023, 909, 875, 816, 787, 735, 647, 571, 516 cm^{-1} ; ^1H NMR (δ) 0.91 (3H, s, H18), 1.06 (3H, s, 16-Me), 1.18 (3H, s, 16-Me), 1.23-2.35 (11H, m), 2.81 (2H, t, $J=4.5$ Hz, H6), 6.56 (1H, d, $J=2.3$ Hz, H4), 6.62 (1H, dd, $J=8.5$ Hz, $J'=2.6$ Hz, H2), 7.09 (1H, d, $J=8.4$ Hz, H1) ppm; ^{13}C NMR (δ) 14.4, 25.7, 25.8, 26.6, 27.1, 29.4, 32.2, 37.5, 37.8, 44.0, 45.3, 47.1, 49.1, 112.7, 115.2, 126.2, 131.3, 137.7, 154.2, 226.3 ppm.

16,16-Dimethyl-2-phenylselenyl-1,3,5(10)-estratrien-3-ol-17-one (19)

15 To a stirred solution of 18 (2.00 g, 6.70 mmol) in MeOH/ CHCl_3 (1:10, 550 mL) at 0°C was added PhSeCl (1.56 g, 8.15 mmol). The reaction mixture was stirred overnight at room temperature. The resulting yellow solution was then poured onto ice/ H_2O (500 mL), extracted with CH_2Cl_2 (2 x 500 mL), dried with MgSO_4 , filtered and then evaporated under reduced pressure. The product was purified by flash chromatography on silica gel using ethyl acetate/toluene (0.3: 9.7) to give 5 as a white foam (2.44 g, 80%) and the 2-chloro isomer (100 mg, 5%). Compound 5: ^1H NMR (δ) 0.94 (3H, s, H18), 1.08 (3H, s, 16-Me), 1.21 (3H, s, 16-Me), 1.24-2.36 (11H, m), 2.92 (2H, dd, $J=8.4$ Hz, $J'=3.6$ Hz, H6), 6.21 (1H, s, OH), 6.82 (1H, s, H4), 7.17-7.23 (5H, m, Ph), 7.54 (1H, s, H1) ppm.

2-Chloro-16,16-dimethyl-1,3,5(10)-estratrien-3-ol-17-one (EM-01831)

To a stirred solution of 5 (680 mg, 1.50 mmol) in anhydrous CHCl_3 (200 mL) at room temperature and under Ar(g) was added N-Chlorosuccinimide (246 mg, 1.84 mmol). The mixture was then stirred at -30°C for one hour. The reaction was quenched with aqueous saturated NH_4Cl (300 mL), extracted with CH_2Cl_2 (2 x 300 mL), dried with Na_2SO_4

5 filtered and then evaporated under reduced pressure. The crude product was purified by flash chromatography using methanol/ethyl acetate/hexane (0.5:1: 9) as the eluent to give EM-01831 as a yellow solid (178 mg, 33%). IR (ν) 3321 (br, s, OH), 2922, 2853, 1724 (CO), 1606, 1502, 1468, 1414, 1380, 1340, 1260, 1215, 1019, 885, 830, 738, 673, 626 cm^{-1} ; ^1H NMR (δ) 0.93 (3H, s, H18), 1.08 (3H, s, 16-Me), 1.21 (3H, s, 16-Me), 1.26-1.35 (11H, m), 2.84 (2H, dd, $J=9.0$, $J'=4.3$ Hz, H6), 5.33 (1H, s, OH), 6.75 (1H, s, H4), 7.20 (1H, s, H1) ppm; ^{13}C NMR (δ) 14.4, 25.7, 26.0, 26.5, 27.3, 29.0, 32.2, 37.5, 37.6, 43.9, 45.3, 47.1, 49.0, 116.0, 117.2, 125.7, 133.4, 137.0, 149.1, 225.0 ppm

15 **2-Chloro-16,16-dimethyl-1,3,5(10)-estratrien-3,17 β -diol (EM-01832)**

To a stirred solution of EM-01831 (200 mg, 0.60 mmol) in anhydrous THF (20 mL) at -78°C under Ar (g) was added LiAlH_4 (33 mg, 0.86 mmol). The temperature of the reaction was allowed to slowly return to rt over 24 hours. The reaction was cooled down to 0°C , more LiAlH_4 (23 mg, 0.60 mmol) was added and the mixture was stirred for another 2 hours. The reaction mixture was quenched with 1M aqueous Rochelle salt (50 mL) then extracted with ethyl acetate (3 x 50 mL). The organic layer was washed with brine (50 mL), dried with MgSO_4 , filtered and then

25 evaporated under reduced pressure. The product was purified by flash chromatography on silica gel using ethyl acetate/toluene (1:19) as eluent to give EM-01832 as white solid (145 mg, 72%). IR (ν) 3557 (s, OH), 3388 (s, OH), 3186 (br, s, OH), 2921, 2861, 1602, 1576, 1486, 1454, 1430, 1409, 1382, 1344, 1256, 1207, 1128, 1069, 1029, 981, 880, 798, 733, 677, 584, 534 cm^{-1} ; ^1H NMR (δ) 0.81 (3H, s, H18), 1.01 (3H, s, 16-Me), 1.06 (3H, s, 16-Me), 1.24-2.20 (11H, m), 2.74 (2H, dd, $J=8.4$ Hz, $J'=3.7$ Hz), 3.23 (1H, s, H17), 6.59 (1H, s, H4), 7.13 (1H, s, H1) ppm; ^{13}C NMR (δ) 11.5, 25.3, 26.2,

30

5 27.2, 29.1, 32.3, 37.7, 37.9, 39.0, 41.2, 43.8, 45.4, 46.8, 89.8, 115.9, 117.0,
125.7, 134.0, 137.3, 148.9 ppm.

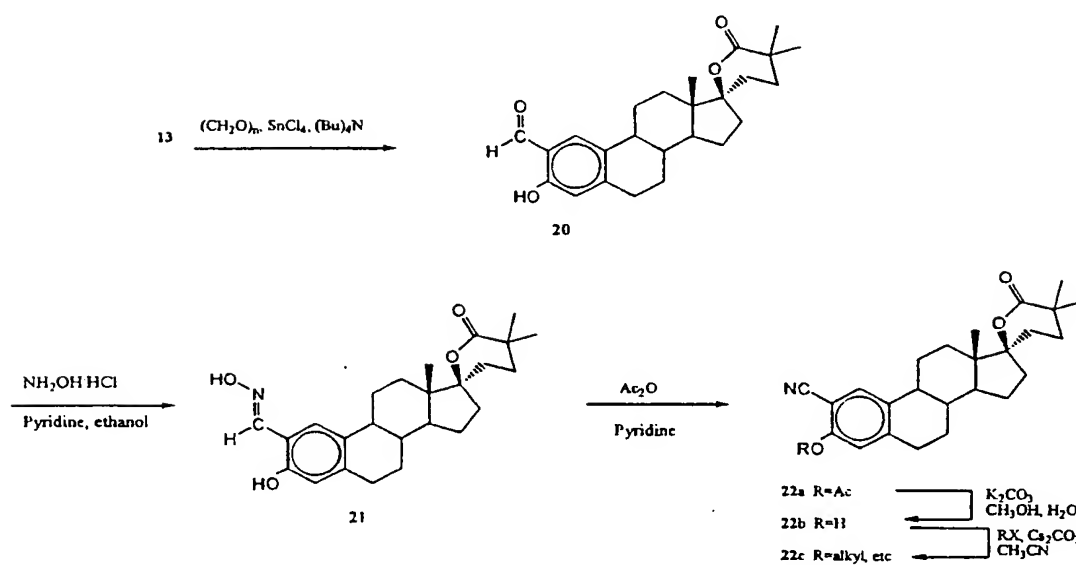
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Example 5

3-hydroxy derivatives of 2-cyano-1,3,5(10)-estratrien-
17-spiro-(dimethyl- δ -lactone)

10

Scheme 5



15

5 Example 5A

2-formyl-1,3,5(10)-estratrien-3-ol-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (20). Lactone 13 (1.0 g, 2.72 mmol) was dissolved in dry 1,2-dichloroethane (9 mL) under argon atmosphere. SnCl₄ (0.16 mL, 1.37 mmol) and Bu₃N (0.52 mL, 2.18 mmol) were added successively. The mixture was stirred at room temperature for 20 min. Formaldehyde (0.23 g, 7.84 mmol) was added and the mixture was stirred at reflux for 6h. The reaction mixture was poured into aq acid (pH=2) and, was extracted with CH₂Cl₂. The organic layers were washed with brine solution, dried (Na₂SO₄) filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel, eluting with (95:5 to 80:20) hexanes-acetone to yield 0.74 g (69 %) of the product; IR (NaCl cm⁻¹): 3164, 2937, 2872, 1716, 1652, 1571, 1487, 1466, 1386, 1298, 1152, 1017, 914, 731; ¹H NMR (CDCl₃) 1.00 (s, 3H), 1.26 (s, 6H) 1.23-2.40 (m, 17H), 2.80-2.90 (m, 2H), 6.66 (s, 1H), 7.39 (s, 1H), 9.79 (s, 1H), 10.77 (s, 1H); ¹³C NMR (CDCl₃) δ 14.3, 23.2, 25.4, 25.9, 26.8, 27.6, 27.7, 30.0, 31.4, 31.6, 34.6, 37.7, 38.6, 42.9, 47.0, 48.5, 93.4, 116.9, 118.9, 130.3, 132.2, 147.8, 159.2, 177.7, 196.0.

25 Example 5B

2-oximino-1,3,5(10)-estratrien-3-ol-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (21). Under argon atmosphere, a solution of compound 20 (215 mg, 0.54 mmol) in anhydrous ethanol-pyridine 1-1 (4 mL) was treated with hydroxylamine hydrochloride (56.6 mg, 0.814 mmol) and stirred at room temperature for 25 min. The reaction mixture was evaporated, diluted with water, and extracted 3 times with dichloromethane. The combined organic phase was washed with brine, dried over sodium sulfate, filtered, and evaporated to provide the oxime 113 (217 mg, 98%); ¹H NMR (300 MHz, CDCl₃) δ 1.02 (s, 3H), 1.29 (s, 6H),

5 1.43-1.70 (m, 10H), 1.89-2.12 (m, 6H), 2.22-2.37 (m, 1H), 2.80-2.87 (m, 2H),
6.69 (s, 1H), 7.05 (s, 1H), 8.15 (broad s, 1H), 8.20 (s, 1H), 9.61 (s, 1H).

Example 5C

10 3-acetoxy-2-cyano-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-
oxo)tetrahydropyran (22a). A solution of compound 21 (180 mg, 0.44
mmol) and acetic anhydride (125 μ L, 1.32 mmol) in pyridine (3.5 mL) was
refluxed for 1 h. The reaction mixture was evaporated, diluted with
dichloromethane, and washed 3 times with water, 1 time with saturated
sodium bicarbonate and 1 time with brine. The organic phase was dried
15 over magnesium sulfate, filtered, and evaporated. The crude mixture
was purified by flash chromatography (dichloromethane to
dichloromethane-ethyl acetate 19-1) to provide acetate 22a (145 mg, 76%):
IR (CHCl₃) 2933, 2872, 2229, 1773, 1718, 1613, 1494, 1183 cm⁻¹; ¹H NMR
(300 MHz, CDCl₃) δ 1.02 (s, 3H), 1.28 (s, 6H), 1.34-1.89 (m, 11H), 1.94-2.33
20 (m, 6H), 2.37 (s, 3H), 2.89-2.94 (m, 2H), 6.95 (s, 1H), 7.55 (s, 1H).

Example 5D

25 2-cyano-1,3,5(10)-estratrien-3-ol-17(R)-spiro-2'-(5',5'-dimethyl-6'-
oxo)tetrahydropyran (22b). A solution of compound 22a (60 mg, 0.14
mmol) in methanol (5 mL) was treated with 10% potassium carbonate
(0.5 mL) and stirred 30 min. The reaction mixture was acidified to pH 2
with 1 N hydrochloric acid and extracted 3 times with dichloromethane.
The combined organic phase was washed with water, saturated sodium
bicarbonate, and brine, dried over magnesium sulfate, filtered, and
30 evaporated. The crude mixture was recrystallized in aqueous ethanol to
afford the phenol 22b (28 mg, 52%). IR (CDCl₃) 3334, 2932, 2868, 1692,
1312, 1206, 1159 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.97 (s, 3H), 1.29 (s,

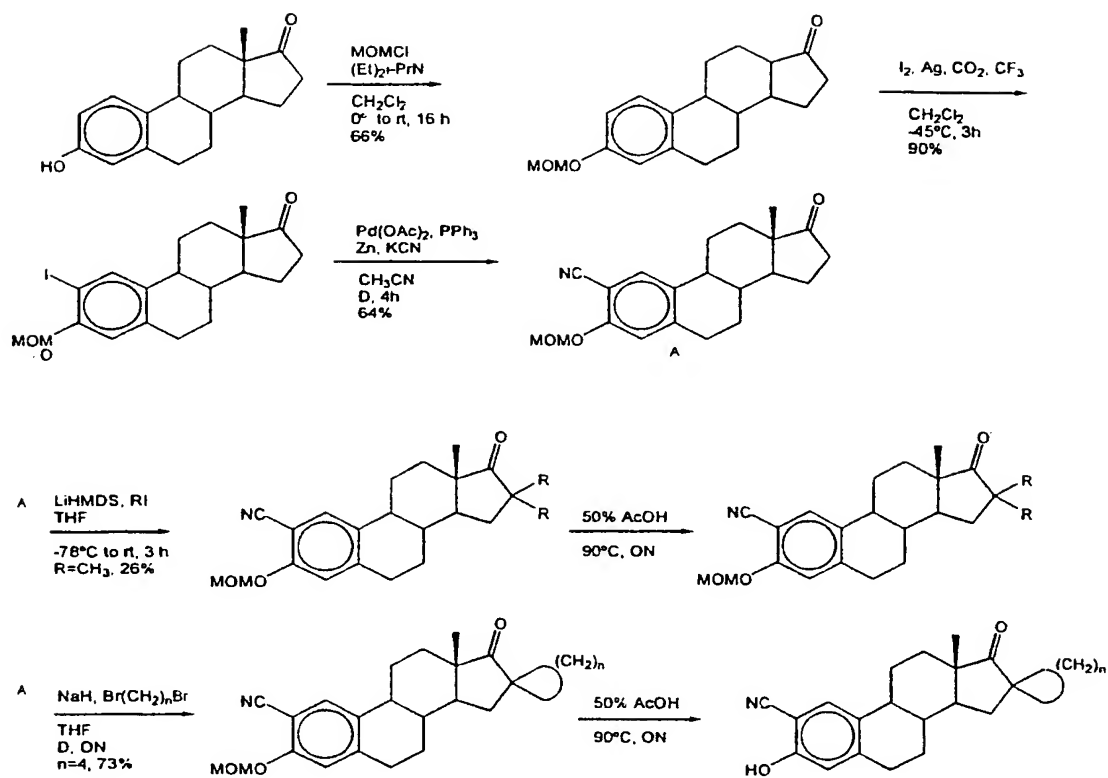
5 6H), 1.26-2.14 (m, 16H), 2.21-2.28 (m, 1H), 2.82-2.86 (m, 2H), 6.69 (s, 1H), 6.91 (s, 1H), 7.35 (s, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.41, 23.30, 25.62, 26.92, 27.69, 27.78, 29.84, 31.62, 31.79, 34.82, 37.84, 38.70, 43.18, 47.23, 48.67, 93.52, 97.01, 116.29, 116.69, 129.44, 133.69, 144.86, 155.73, 177.88.

10 **Example 5E**

3-alkyloxy-2-cyano-1,3,5(10)-estratrien-17(R)-spiro-2'-(5',5'-dimethyl-6'-oxo)tetrahydropyran (22c). Under argon atmosphere, a suspension of compound 22b, alkyl iodide (5 equiv) and cesium carbonate (1.5 equiv) in anhydrous acetonitrile (1% W/V) was stirred for 16 h with refluxing condition if necessary. The reaction mixture was quenched with brine and extracted 3 times with dichloromethane. The combined organic phase was washed with brine, dried over magnesium sulfate, filtered, and evaporated. The crude mixture was purified by flash chromatography (dichloromethane to dichloromethane-ethyl acetate 10-1) and recrystallization (methanol) to provide compound 22c (e.g., EM-1396, R=(CH₂)₂OCH₃, 75%): IR (CHCl_3) 3013, 2941, 2881, 2229, 1710, 1610, 1500, 1304, 1136 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 1.01 (s, 3H), 1.28 (s, 6H), 1.20-1.75 (m, 10H), 1.80-2.20 (m, 6H), 2.20-2.35 (m, 1H), 2.80-2.95 (m, 2H), 3.47 (s, 3H), 3.79 (t, $J=4.8$ Hz, 2H), 4.17 (t, $J=4.8$ Hz, 2H), 6.67 (s, 1H), 7.44 (s, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.41, 23.25, 25.58, 25.90, 26.96, 27.66, 27.74, 30.24, 31.59, 31.78, 34.79, 37.80, 38.68, 43.16, 47.20, 48.60, 59.55, 68.78, 70.71, 93.43, 99.55, 112.80, 116.98, 130.72, 133.31, 144.09, 158.29, 177.70.

5

Example 6



5

PHARMACEUTICAL COMPOSITION EXAMPLES

10

Set forth below, by way of example and not of limitation, are several pharmaceutical compositions utilizing a preferred active compound EM-2330 (an inhibitor of type 3 3α -HSD). Other compounds of the invention or combination thereof may be used in place of (or in addition to) EM-02318 and EM-02200. The concentration of active ingredient may be varied over a wide range compatible with the preferred dosages discussed herein, and depending on preferred frequency of administration. The amounts and types of other ingredients that may be included are well known in the art.

15

EXAMPLE A

Composition suitable for injection

Ingredient	Weight % (by weight of total composition)
EM-2330	0.4
Ethanol	6.4
NaCl	0.8
Water	91.5
Benzyl alcohol	0.9

20

5

EXAMPLE B

Composition suitable for use as topical lotion

Ingredient	Weight % (by weight of total composition)
EM-2330	1.0
Ethanol	70.0
Propylene glycol	29.0

EXAMPLE C

10

Composition suitable for use as topical gel

Ingredient	Weight % (by weight of total composition)
EM-2330	1.0
Hydroxypropylcellulose	1.5
Ethanol	70.0
Propylene glycol	27.5

EXAMPLE D

Tablet

15

Ingredient	Weight % (be weight of total composition)
EM-2330	1.0
Gelatin	5.0
Lactose	67.5
Starch	26.5

5

EXAMPLE E
Gelatin capsule

Ingredient	Weight % (be weight of total composition)
EM-2330	2.0
Lactose hydrous	80.0
Starch	4.8
Cellulose microcrystalline	12.8
Magnesium atearate	0.4

10

EXAMPLE F
Composition suitable for use as topical gel

Ingredient	Weight % (be weight of total composition)
EM-2330	1.0
Ethanol	4.0
Polyethylene glycol	4.0
Gelatin	1.0
NaCl	0.9
Benzyl alcohol	1.0
Water USP	88.1

15

Other inhibitors of type 3 3α -hydroxysteroid dehydrogenase may be substituted for EM-2330 in the above formulations. In some embodiments, two or more type 3 3α -hydroxysteroid inhibitors may be

5 included together, (or one inhibitor of type 3 3α -HSD plus one inhibitor of
type 5 17β -HSD) in which case the combined weight percent of the two is
preferably double what is shown in the above examples for EM-2330
alone, with a corresponding reduction in the weight percent of the most
prevalent excipient (e.g., water, lactose, ethanol or the like). Other active
10 ingredients of preferred combinations herein may be added in like
manner.

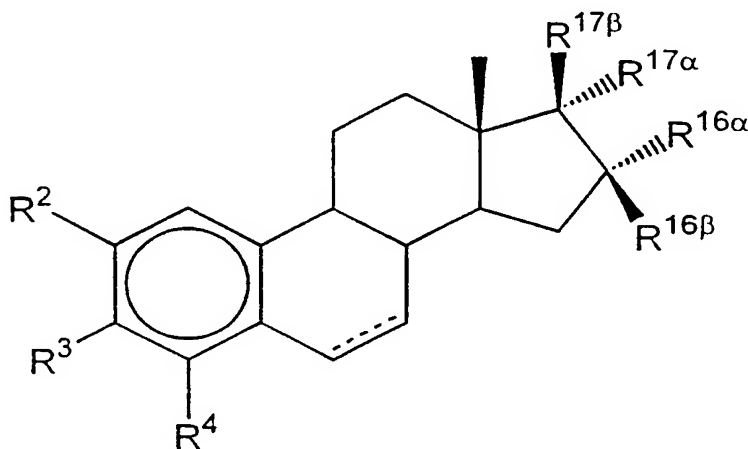
The invention has been described in terms of preferred embodiments and
examples, but is not limited thereby. Those of skill in the art will readily
15 recognize the broader applicability and scope of the invention which is
limited only by the patent claims herein.

5 What is claimed is:

1. A method of inhibiting conversion of 4-androstene-3,17-dione to testosterone or of 5 α -androstane-3,17-dione to dihydrotestosterone in a patient in need of such inhibition comprising
10 administering to said patient a therapeutically effective amount of an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase.

2. The method of claim 1 further comprising administering an
15 inhibitor of type 5 17 β -hydroxysteroid dehydrogenase.

3. A method of inhibiting activity of human type 3, 3 α -hydroxysteroid dehydrogenase comprising administering to a patient in need of such treatment a therapeutically effective amount of an
20 inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase having the following structure:



5 wherein the dotted line is an optional pi bond;

 wherein R³ is a moiety selected from the group consisting of C₁-C₂₀ alkyloxy, C₁-C₁₀ acyloxy, C₁-C₂₀ alkoxy carbonyloxy, C₁-C₂₀ alkyloxy alkyloxy, hydroxyl, (N-alkyl or -H) carbamate and a moiety transformed in vivo to hydroxyl;

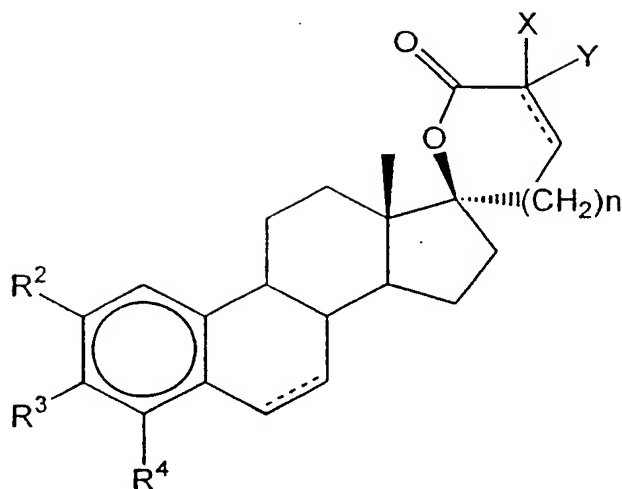
10 wherein R² and R⁴ are independently selected from the group consisting of hydrogen, cyano, fluoro, chloro, bromo, and nitro (wherein R² and R⁴ are not simultaneously hydrogen).

 wherein R^{17a} is selected from the group consisting of hydrogen, a C₂-C₁₄ carbon moiety substituted by a radical selected from the group
15 consisting of hydrogen, halogen carboxyl, amido, C₁-C₃ alkoxy and C₁-C₅ alkyl or R^{17a} and R^{17b} together form a C₅-C₇ lactone ring or is a ketonic oxygen;

 wherein R^{17b} is hydroxyl acyloxy, alkoxy, alkenyloxy, (N-alkyl or H) amido; or R^{17a} and R^{17b} together form a C₅-C₇ lactone ring or is a ketonic
20 oxygen;

 wherein R^{16a} and R^{16b} are independently selected from the group consisting of hydrogen, lower alkyl, and benzyl, or R^{16a} and R^{16b} together form a C₅-C₆ cycloalkene.

25 4. The method of claim 3 wherein the inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase has the molecular structure:



5

wherein n is an integer from 1-2;

wherein the dotted lines are independently optional pi bonds;

wherein X and Y are independently selected from the group consisting of H , (C_1-C_3) alkyl, and (C_2-C_3) alkenyl.

10

5. The method of Claim 4 wherein R^3 is hydroxy.

6. The method of Claim 4 wherein at least one of X , or Y is methyl.

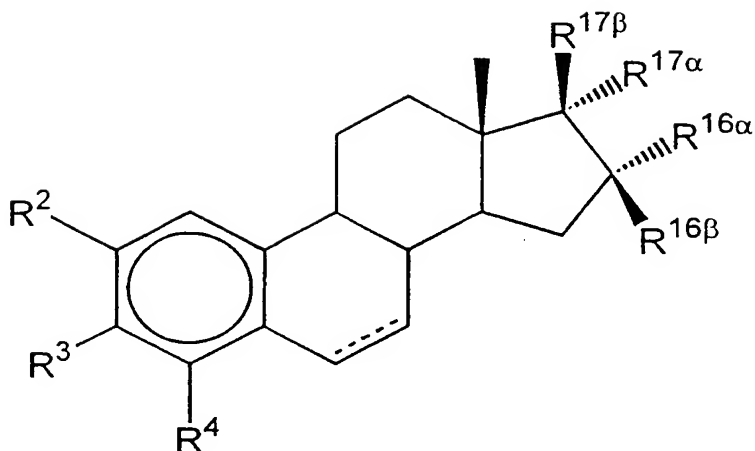
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7. The method of Claim 4 wherein both X and Y are methyl.

8. The method of claim 4 wherein R_2 is chlorine or cyano.

20

9. The method of claim 3 wherein the inhibitor of human type 3 3α -hydroxysteroid dehydrogenase has the molecular structure:



wherein either:

- i) $R^{17\beta}$ is an hydroxyl, $R^{17\alpha}$ is C_2 - C_{14} carbon moiety substituted by a radical selected from the group consisting of hydrogen halogen, carboxyl, amido, C_1 - C_3 alkoxy, and C_1 - C_5 alkyl, and $R^{16\alpha}$ and $R^{16\beta}$ are hydrogen; or
- ii) $R^{17\beta}$ is hydroxyl, $R^{17\alpha}$ is hydrogen and $R^{16\alpha}$ and $R^{16\beta}$ are either lower alkyl, benzyl or are together a C_5 - C_6 cycloalkane; or
- iii) $R^{17\alpha}$ and $R^{17\beta}$ are together a ketonic oxygen and $R^{16\alpha}$ and $R^{16\beta}$ are either lower alkyl, benzyl, or are together a C_5 - C_6 cycloalkane.

10. The method of Claim 9 wherein $R^{17\alpha}$ is a benzyl group.

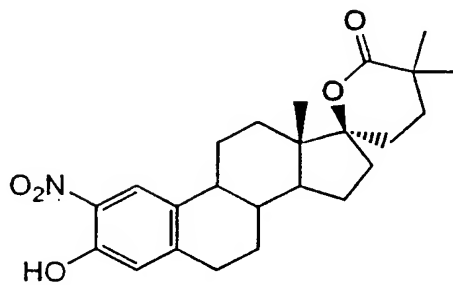
11. The method of Claim 10 wherein R^3 is hydroxy.

12. The method of Claim 10 wherein R^2 is chlorine or cyano.

13. A method of inhibiting the activity of human type 3 3α -hydroxysteroid dehydrogenase comprising administering to a patient in need of such inhibitor a therapeutically effective amount of an

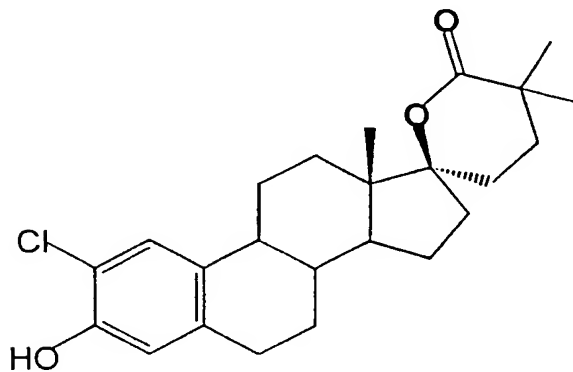
- 5 inhibitor of human type 3 3α -hydroxysteroid dehydrogenase selected from the group consisting of:

EM-1125

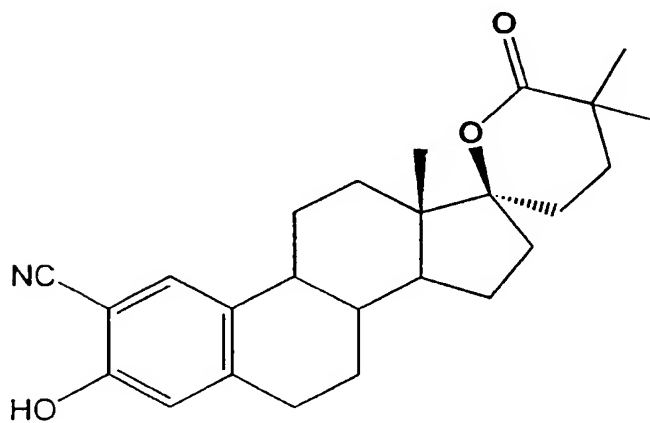


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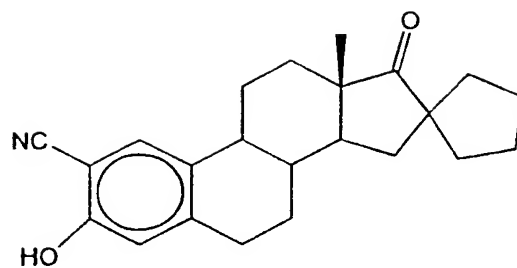
EM-01667-C



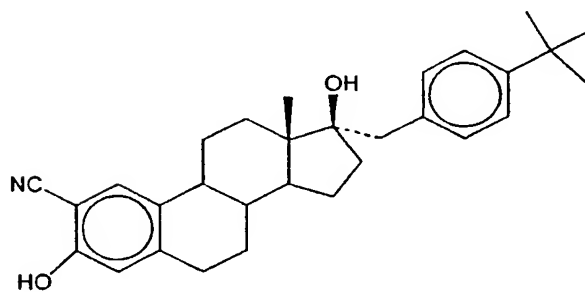
EM-01645



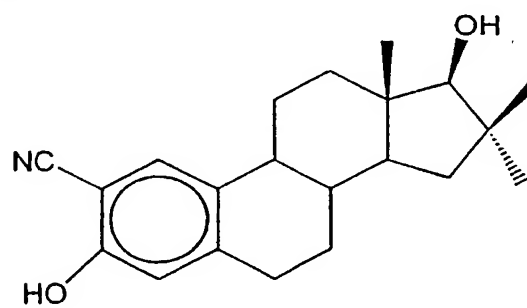
EM-1834



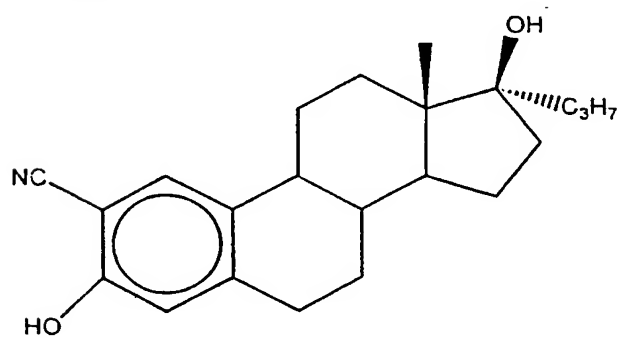
EM-2359



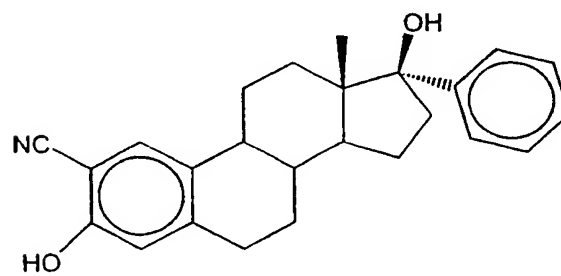
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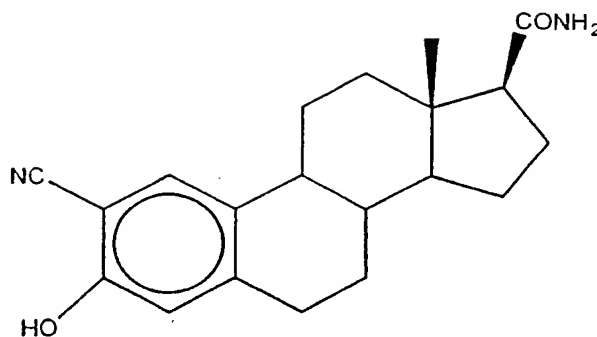
EM-2132



EM-2318



EM-2330



5

14. A method for determining effectiveness of a putative inhibitor of the conversion of 4-androstene-3,17-dione to testosterone and 5 α -androstane-3,17-dione to dihydrotestosterone, comprising measuring activity of type 3 3 α -hydroxysteroid dehydrogenase in the presence of said putative inhibitor and correlating effectiveness to a reduction in said activity relative to activity of said dehydrogenase in the absence of said putative inhibitor.

15

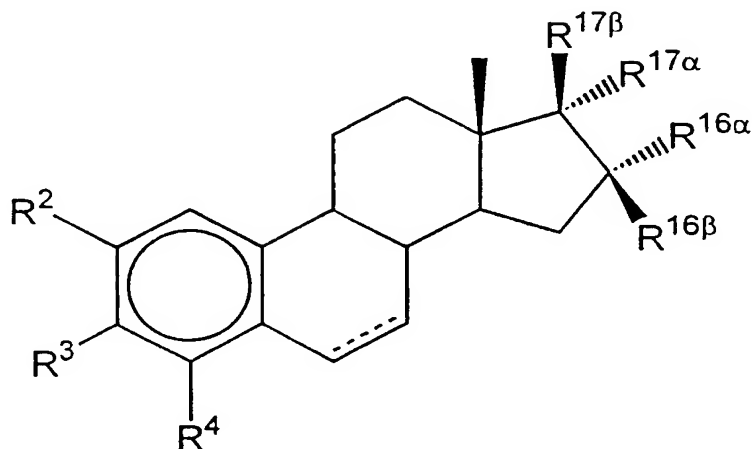
15. The method of claim 14 wherein said method comprises the following steps :

- a) providing culture media with recombinant host cells transformed or transfected with a recombinant vector comprising a promoter sequence and a nucleotide sequence encoding human type 3 3 α -hydroxydehydrogenase;
- b) providing to said media both said putative inhibitor and a substrate that , in the absence of inhibitor, undergoes a type 3 3 α -Human Hydroxysteroid Dehydrogenase conversion ; and
- c) measuring said conversion.

25

5 16 A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically effective amount of an inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase having the molecular structure:

10



wherein R³ is a moiety selected from the group consisting of C₁-C₂₀ alkyloxy, C₁-C₁₀ acyloxy, C₁-C₂₀ alkoxycarbonyloxy, C₁-C₂₀ alkyloxy alkyloxy, hydroxyl, (N-alkyl or -H) carbamate and a moiety transformed in vivo to hydroxyl;

15

wherein R² and R⁴ are independently selected from the group consisting of hydrogen, cyano, fluoro, chloro, bromo, and nitro (wherein R² and R⁴ are not simultaneously hydrogen).

wherein the dotted line is an optional pi bond;

20

wherein R^{17 α} is selected from the group consisting of hydrogen, a C₂-C₁₄ carbon moiety substituted by a radical selected from the group consisting of hydrogen, halogen, carboxyl, amido, C₁-C₃ alkoxy and C₁-C₅ alkyl or R^{17 α} and R^{17 β} together form a C₅-C₇ lactone ring or is a ketonic oxygen;

5 wherein R^{17b} is selected from the group consisting of hydroxyl, acyloxy, alkoxy, alkenyloxy, (N-alkyl or H) amido; or R^{17a} and R^{17b} together form a C₅-C₇ lactone ring or is a ketonic oxygen;

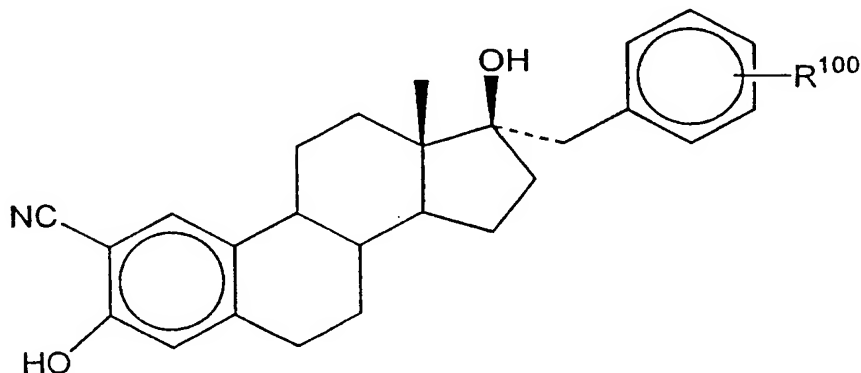
10 17. The pharmaceutical composition of Claim 16 wherein R^{17a} is a phenyl or propyl group.

18. The pharmaceutical composition of Claim 16 wherein R^3 is hydroxy.

15 19. The pharmaceutical composition of Claim 16 wherein R_2 is chlorine or cyano.

20 20. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of human type 3 3α -hydroxysteroid dehydrogenase having the molecular structure:

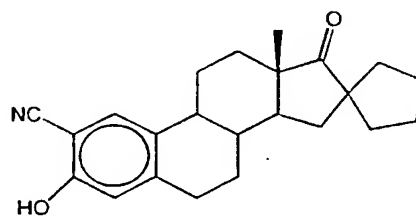
wherein R^{100} is selected from the group consisting of hydrogen,



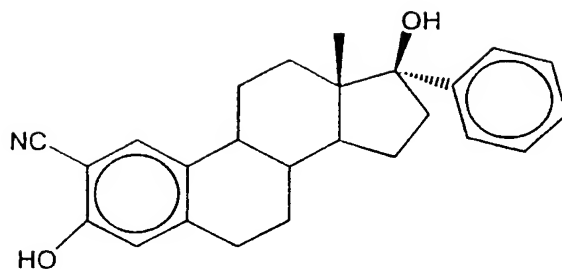
carboxyl, amido, C₁-C₅ alkyl, halo, nitro, hydroxy, and C₁-C₃ alkoxy.

- 5 21. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a therapeutically acceptable amount of an inhibitor of human type 3 3α -hydroxysteroid dehydrogenase selected from the group consisting of

EM-1834

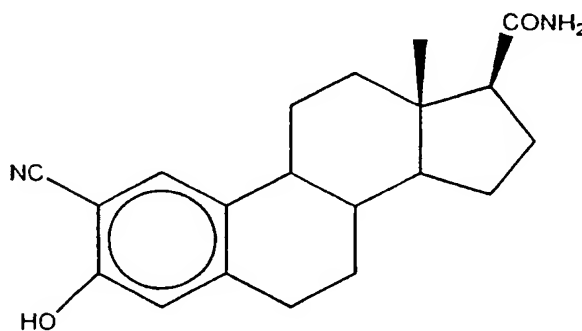


EM-2318



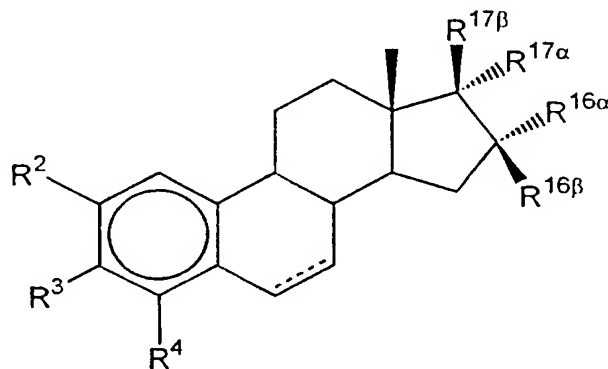
and

EM-2330



5

22. An inhibitor of human type 3 3α -hydroxysteroid dehydrogenase having the molecular structure:



10 wherein R^3 is a moiety selected from the group consisting of C_1 - C_{20} alkyloxy, C_1 - C_{10} acyloxy, C_1 - C_{20} alkoxy carbonyloxy, C_1 - C_{20} alkyloxy alkyloxy, hydroxyl; (N-alkyl or -H) carbamate and a moiety transformed in vivo to hydroxyl;

15 wherein R^2 and R^4 are independently selected from the group consisting of hydrogen, cyano, fluoro, chloro, bromo, and nitro (wherein R^2 and R^4 are not simultaneously hydrogen).

5 wherein the dotted line is an optional pi bond;

 wherein R^{17a} is selected from the group consisting of hydrogen, a C_2-C_{14} carbon moiety substituted by a radical selected from the group consisting of hydrogen, halogen, carboxyl, amido, C_1-C_3 alkoxy and C_1-C_5 alkyl or R^{17a} and R^{17b} together form a C_5-C_7 lactone ring or is a ketonic oxygen;

10

 wherein R^{17b} is selected from the group consisting of hydroxyl, acyloxy, alkoxy, alkenyloxy, (N-alkyl or H) amido; or R^{17a} and R^{17b} together form a C_5-C_7 lactone ring or is a ketonic oxygen;

 wherein R^{16a} and R^{16b} are independently selected from the group consisting of hydrogen, lower alkyl, and benzyl, or R^{16a} and R^{16b} together form a C_5-C_6 cycloalkene.

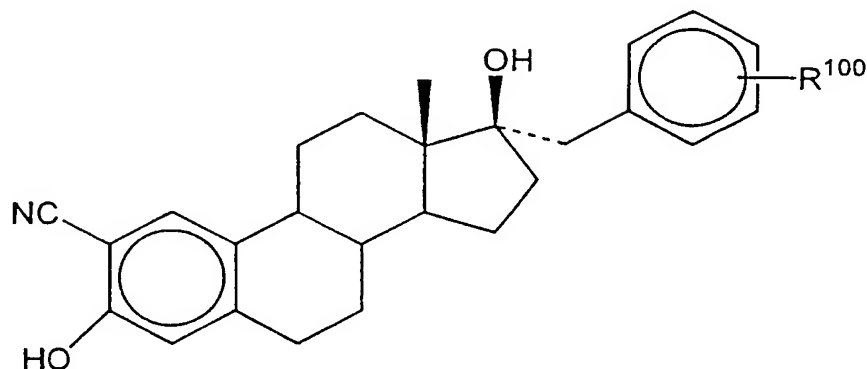
15

23. The inhibitor of Claim 22 wherein R^{17a} is a phenyl or propyl group.

20 24. The inhibitor of Claim 22 wherein R^3 is hydroxy.

25. The inhibitor of Claim 22 wherein R_2 is chlorine or cyano.

5 26. An inhibitor of human type 3 3 α -hydroxysteroid dehydrogenase having the molecular structure:



wherein R¹⁰⁰ is selected from the group consisting of hydrogen, carboxyl, amido, C₁-C₅ alkyl, halo, nitro, hydroxy, and C₁-C₃ alkoxy.

10 27. A method of treating, or reducing the risk of developing prostate cancer, comprising administering to a patient in need of such treatment or reduction a therapeutically effective amount of an inhibitor of the 17 β - hydroxysteroid dehydrogenase activity of human type 3 3 α -hydroxysteroid dehydrogenase other than 17-lactone derivative compounds.

15 28. The method of claim 27 further comprising administering a therapeutically effective amount of an inhibitor of human type 5 17 β -hydroxysteroid dehydrogenase.

20 29. The method of claim 27 wherein prostate cancer is treated and further comprising administering a therapeutically effective amount of an LHRH agonist (or antagonist) effective to reduce testicular secretion of sex steroids.

25

5

30. The method of claim 28 wherein prostate cancer is treated, said inhibitor further comprising administering a therapeutically effective amount of an LHRH agonist (or antagonist) effective to reduce testicular secretion of sex steroids.

10

31. The method of claim 29, further comprising administering a therapeutically effective amount of an antiandrogen.

15

32. The method of claim 30, further comprising administering a therapeutically effective amount of an antiandrogen.

33. The method of claim 27, further comprising administering a therapeutically effective amount of a 5 α -reductase inhibitor.

20

34. The method of claim 28, further comprising administering a therapeutically effective amount of a 5 α -reductase inhibitor.

35. The method of claim 29, further comprising administering a therapeutically effective amount of a 5 α -reductase inhibitor.

25

36. The method of claim 30, further comprising administering a therapeutically effective amount of a 5 α -reductase inhibitor.

30

37. The method of claim 31, further comprising a therapeutically effective amount of a 5 α -reductase inhibitor.

5 38. The method of claim 32, further comprising a
therapeutically effective amount of a 5 α -reductase inhibitor.

 39. The method of claim 27, further comprising a
therapeutically effective amount of an type 3 17 β -hydroxysteroid
10 dehydrogenase.

 40. The method of claim 28, further comprising a
therapeutically effective amount of an type 3 17 β -hydroxysteroid
dehydrogenase.

15 41. The method of claim 29, further comprising a
therapeutically effective amount of a type 3 17 β -hydroxysteroid
dehydrogenase.

20 42. The method of claim 30, further comprising a
therapeutically effective amount of a type 3 17 β -hydroxysteroid
dehydrogenase.

 43. The method of claim 31, further comprising a
25 therapeutically effective amount of a type 3 17 β -hydroxysteroid
dehydrogenase.

 44. The method of claim 32, further comprising a
therapeutically effective amount of a type 3 17 β -hydroxysteroid
30 dehydrogenase.

5 45. The method of claim 35, further comprising a
therapeutically effective amount of a type 3 17 β -hydroxysteroid
dehydrogenase.

10 46. The method of claim 36, further comprising a
therapeutically effective amount of a type 3 17 β -hydroxysteroid
dehydrogenase.

15 47. The method of claim 37, further comprising a
therapeutically effective amount of a type 3 17 β -hydroxysteroid
dehydrogenase.

20 48. The method of claim 38, further comprising a
therapeutically effective amount of a type 3 17 β -hydroxysteroid
dehydrogenase.

 49. The method of claim 27, further comprising a
therapeutically effective amount of an antiandrogen.

25 50. A method of treating, or reducing the risk of developing,
benign prostatic hyperplasia comprising administering to a patient in
need of such treatment or reduction, a therapeutically effective amount of
an inhibitor of the 17 β -hydroxysteroid dehydroxygenase activity of
human type 3 3 α -hydroxysteroid dehydrogenase other than
administering a 17-lactone derivative compound.

30

5 51. The method of claim 50 further comprising administering to
said patient therapeutically effective amount of an inhibitor of human
type 5 17β -hydroxysteroid dehydrogenase.

10 52. The method of claim 50, further comprising administering
to said patient a therapeutically effective amount of an agent selected
from the group consisting of an antiestrogen or an aromatase inhibitor.

15 53. The method of claim 51, further comprising administering
to said patient a therapeutically effective amount of an agent selected
from the group consisting of an antiestrogen or an aromatase inhibitor.

 54. The method of claim 52, further comprising administering
to said patient a therapeutically effective amount of an antiandrogen.

20 55. The method of claim 53, further comprising administering
to said patient a therapeutically effective amount of an antiandrogen.

25 56. The method of claim 54, further comprising administering
to said patient a therapeutically effective amount a 5α -reductase
inhibitor.

 57. The method of claim 55, further comprising administering
to said patient a therapeutically effective amount a 5α -reductase
inhibitor.

5 58. The method of claim 52, further comprising administering
to said patient a therapeutically effective amount of a 5 α -reductase
inhibitor.

10 59. The method of claim 53, further comprising administering
to said patient a therapeutically effective amount of a 5 α -reductase
inhibitor.

15 60. The method of claim 52, further comprising administering
to said patient a therapeutically effective amount of a 5 α -reductase
inhibitor and of an antiestrogen or an aromatase inhibitor.

20 61. The method of claim 53, further comprising administering
to said patient a therapeutically effective amount of a 5 α -reductase
inhibitor and of an antiestrogen or an aromatase inhibitor.

25 62. The method of claim 52, further comprising administering
to said patient a therapeutically effective amount of a 5 α -reductase
inhibitor, an antiandrogen, and of an antiestrogen or an aromatase
inhibitor.

30 63. The method of claim 53, further comprising administering
to said patient a therapeutically effective amount of a 5 α -reductase
inhibitor, an antiandrogen, and of an antiestrogen or an aromatase
inhibitor.

5 64. A method of treating, or reducing the risk of developing, prostatitis comprising administering to a patient in need of such treatment or reduction, a therapeutically effective amount of an inhibitor of the 17β -hydroxysteroid dehydrogenase activity of human type 3 3α -hydroxysteroid dehydrogenase.

10 65. The method of claim 64 further comprising administering a therapeutically effective amount of an inhibitor of human type 5 17β -hydroxysteroid dehydrogenase.

15 66. The method of claim 64, further comprising administering to said patient a therapeutically effective amount of an antiandrogen.

 67. The method of claim 65, further comprising administering to said patient a therapeutically effective amount of an antiandrogen.

20 68. The method of claim 64, further comprising administering to said patient a therapeutically effective amount a 5α -reductase inhibitor.

25 69. The method of claim 65, further comprising administering to said patient a therapeutically effective amount a 5α -reductase inhibitor.

30 70. The method of claim 66, further comprising administering to said patient a therapeutically effective amount of a 5α -reductase inhibitor.

5 71. The method of claim 67, further comprising administering
to said patient a therapeutically effective amount of a 5α -reductase
inhibitor.

10 72. A method of treating or reducing the risk of developping
acne, seborrhea, hirsutism or androgenic alopecia comprising
administering to a said patient, in need of such treatment or reduction, a
therapeutically effective amount of an inhibitor of human type 5 17β -
hydroxysteroid dehydrogenase activity of human type 3 3α -
15 hydroxysteroid dehydrogenase other than by administering a 17-lactone
derivative compound.

 73. The method of claim 72, further comprising administering
to said patient a therapeutically effective amount of an inhibitor of
human type 5 17β -hydroxysteroid dehydrogenase.

20 74. The method of claim 72, further comprising administering
to said patient a therapeutically effective amount of an antiandrogen.

 75. The method of claim 73, further comprising administering
25 to said patient a therapeutically effective amount of an antiandrogen.

 76. The method of claim 72, further comprising administering
to said patient a therapeutically effective amount of 5α -reductase
inhibitor.

30

5 77. The method of claim 73, further comprising administering
to said patient a therapeutically effective amount of 5 α -reductase
inhibitor.

10 78. The method of claim 74, further comprising administering
to said patient a therapeutically effective amount of 5 α -reductase
inhibitor.

15 79. The method of claim 75, further comprising administering
to said patient a therapeutically effective amount of 5 α -reductase
inhibitor.

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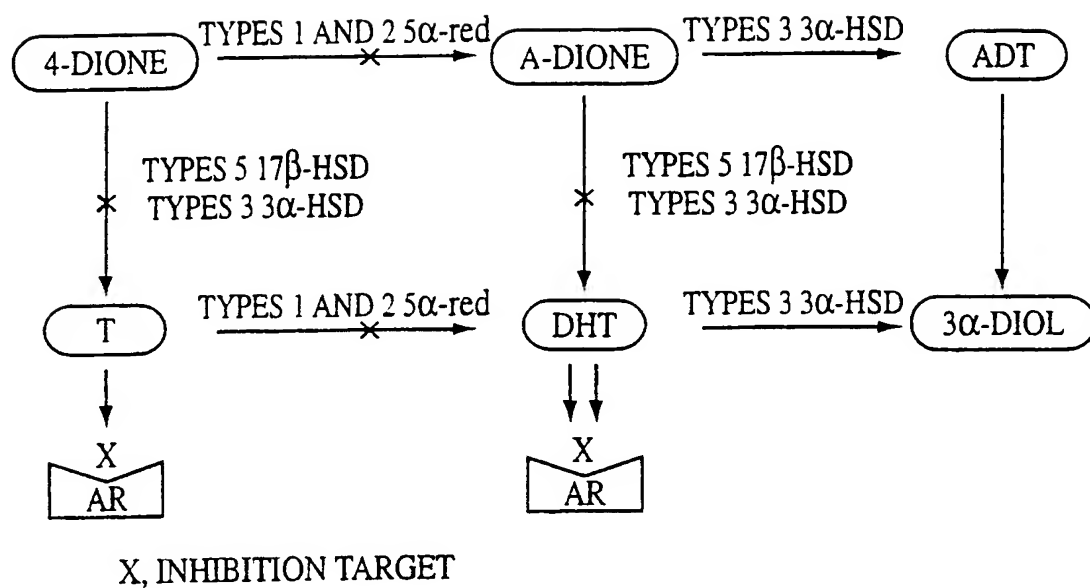


FIG. 1

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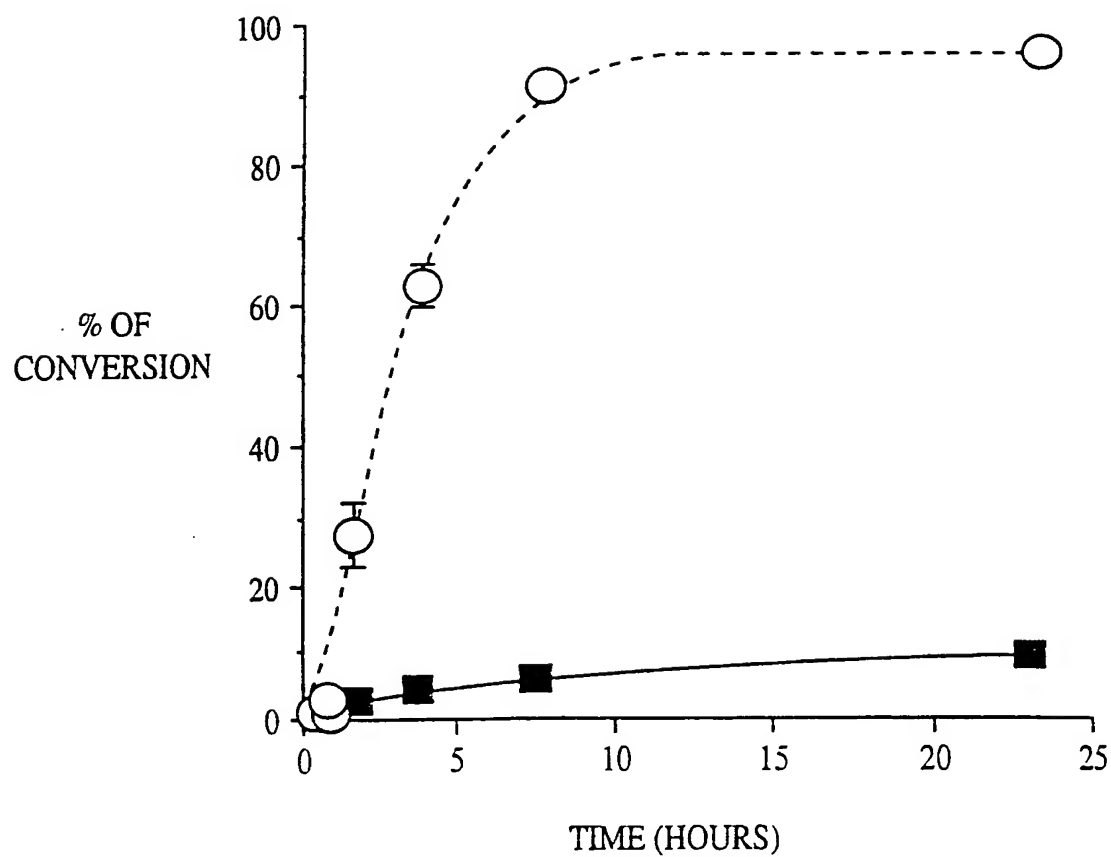


FIG. 2

FIG. 3A



FIG. 3B



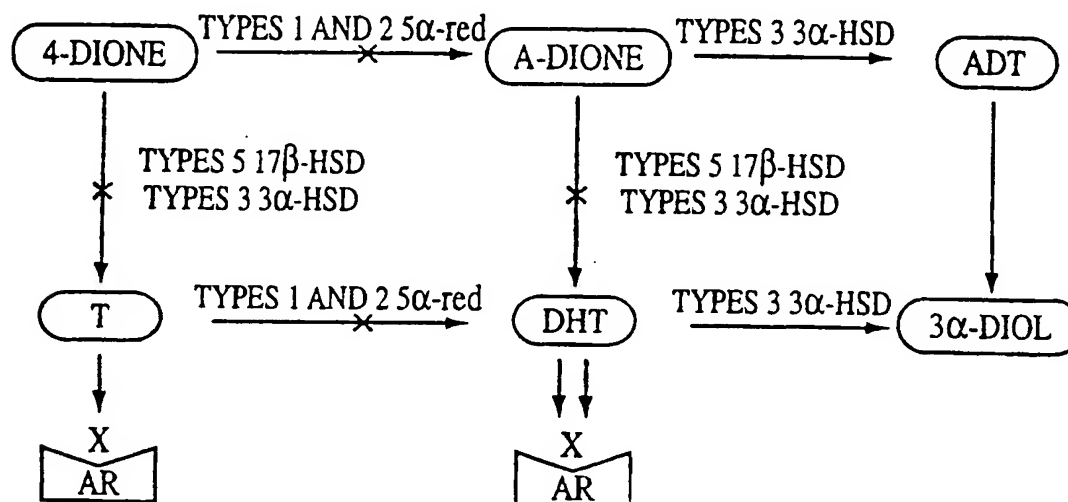
FIG. 3C





INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/00, 31/565, 31/566, 31/567, 31/585, 45/06, A61P 35/00, 17/10, 17/08, 17/14, 5/26		A3	(11) International Publication Number: WO 00/07576
			(43) International Publication Date: 17 February 2000 (17.02.00)
(21) International Application Number: PCT/CA99/00724		Charny, Québec G6X 3R7 (CA). LUU-THE, Van [CA/CA]; 4460 de l'Estuaire, Charny, Québec G6X 1C6 (CA).	
(22) International Filing Date: 6 August 1999 (06.08.99)		(74) Agent: MITCHELL, Richard, J.; Marks & Clerk, P.O. Box 957, Station B, Ottawa, Ontario K1P 5S7 (CA).	
(30) Priority Data: 60/095,623 7 August 1998 (07.08.98) US		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/095,623 (CON) Filed on 7 August 1998 (07.08.98)		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
(71) Applicant (for all designated States except US): EN-DORECHER, INC. [CA/CA]; 2989 de la Promenade, Ste-Foy, Québec G1W 2J5 (CA).		(88) Date of publication of the international search report: 30 March 2000 (30.03.00)	
(72) Inventors; and (75) Inventors/Applicants (for US only): LABRIE, Fernand [CA/CA]; 2989 de la Promenade, Ste-Foy, Québec G1W 2J5 (CA). MERAND, Yves [CA/CA]; 3101 Montreux, Sainte-Foy, Québec G1W 3A1 (CA). GAUTHIER, Sylvain [CA/CA]; 152, rue de la Modiste, Saint-Augustin-de-Desmaures, Québec G3A 2L2 (CA). PROVENCHER, Louis [CA/CA]; 7518 de la Sarcelle,			

(54) Title: INHIBITORS OF TYPE 3 3 α -HYDROSTEROID DEHYDROGENASE

X, INHIBITION TARGET

(57) Abstract

Novel methods of treating and/or inhibiting development of prostatic cancer, benign prostatic hyperplasia, prostatitis, acne, seborrhea, hirsutism or androgenic alopecia utilize inhibitors of type 3 3 α -hydroxysteroid dehydrogenase alone or in combination with other active pharmaceuticals such as inhibitors of type 5 17 β -hydroxysteroid dehydrogenase. Novel inhibitors and pharmaceutical products are also disclosed.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 99/00724

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/00 A61K31/565 A61K31/566 A61K31/567 A61K31/585
 A61K45/06 A61P35/00 A61P17/10 A61P17/08 A61P17/14
 A61P5/26

According to International Patent Classification: (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 99 46279 A (ENDORECHERCHE INC) 16 September 1999 (1999-09-16) abstract; example 30; table 4 claims 19-25, 32, 36, 58-63, 71, 93-98, 106, 114-204 ---	1-19, 22-25, 27-79
X	US 3 562 260 A (RUGGIERI PIETRO DE ET AL) 9 February 1971 (1971-02-09) column 1, line 15 -column 2, line 12; claims; examples 8-16 --- -/--	1-3, 9, 11-13, 16-19, 21-25, 27-79



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

25 January 2000

Date of mailing of the international search report

02/02/2000

Name and mailing address of the ISA

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Authorized officer

Hoff, P

INTERNATIONAL SEARCH REPORT

Int .tional Application No

PCT/CA 99/00724

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 117 117 A (LIEHR JOACHIM GEORG) 29 August 1984 (1984-08-29) the whole document ----	16, 18, 19, 22, 24, 25
X	WO 95 04535 A (CHILDRENS MEDICAL CENTER) 16 February 1995 (1995-02-16) abstract page 6, line 21 -page 7, line 2; example 4; table 1 claims 1, 14, 15 ----	16, 18, 19, 22, 24, 25
X	PAGE, PHILIP C. BULMAN ET AL: "Efficient regioselective A-ring functionalization of estrogens" TETRAHEDRON (1990), 46(6), 2059-68 , XP000867284 the whole document ----	22, 24, 25
X	US 4 340 602 A (BROOKS SAMUEL C) 20 July 1982 (1982-07-20) the whole document ----	16, 22
X	US 4 568 673 A (BROOKS SAMUEL C ET AL) 4 February 1986 (1986-02-04) the whole document ----	16, 22
A	DUFORT, ISABELLE ET AL: "Molecular cloning of human type 3 3.alpha.-hydroxysteroid dehydrogenase that differs from 20.alpha.-hydroxysteroid dehydrogenase by seven amino acids" BIOCHEM. BIOPHYS. RES. COMMUN. (1996), 228(2), 474-479 , XP000867346 the whole document ----	14, 15
A	LUU-THE V ET AL: "CHARACTERISTICS OF HUMAN TYPES 1, 2 AND 3 17BETA-HYDROXYSTEROID DEHYDROGENASE ACTIVITIES: OXIDATION/REDUCTION AND INHIBITION" JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, GB, ELSEVIER SCIENCE LTD., OXFORD, vol. 55, no. 5/06, page 581-587 XP000196678 ISSN: 0960-0760 abstract; figure 5 -----	1-19, 22-25, 27-79

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 99/00724

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-13, 27-79
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
SEE FURTHER INFORMATION PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-2,27-79 relate to compounds defined by reference to their pharmacological properties, namely "type 3 3alpha-hydroxysteroid dehydrogenase inhibitor", "types 5/3 17beta-hydroxysteroid dehydrogenase inhibitor", "LHRH agonist/antagonist", "antiandrogen", "5alpha-reductase inhibitor", "antiestrogen", "aromatase inhibitor".

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by their pharmacological profiles. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Furthermore, claims 1-13 relate to the treatment of a disease which actually is not well defined. The use of the definition "inhibiting conversion of 4-androstene-3,17-dione/ 5alpha-androstane-3,17-dione to testosterone/dihydrotestosterone" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. The lack of clarity is such as to render a meaningful complete search impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds structurally identified in claims 3-13,16-26, to the diseases explicitly mentioned in claims 27,50,64,72 and to the general idea underlying the present invention.

Claims searched completely: 3-13,16-26

Claims searched incompletely: 1,2,14,15,27-79

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 99/00724

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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